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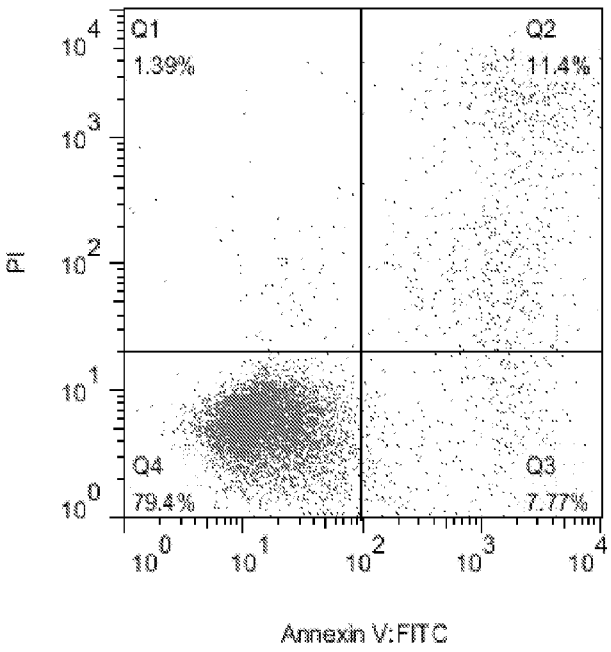
[Continued on next page]

(54) Title: ANTI-CD38 ANTIBODIES FOR TREATMENT OF ACUTE MYELOID LEUKEMIA

Figure 1A.



(57) Abstract: The present invention relates to methods of treatment of acute myeloid leukemia with anti- CD38 antibodies.

WO 2016/089960 A1

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ANTI-CD38 ANTIBODIES FOR TREATMENT OF ACUTE MYELOID LEUKEMIA

FIELD OF THE INVENTION

The present invention relates to methods of treatment of acute myeloid leukemia with anti-CD38 antibodies.

BACKGROUND OF THE INVENTION

CD38 is a type II membrane protein with ADP ribosyl cyclase activity, catalyzing formation of second messengers cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP) from NAD and NADP, respectively. CD38 mediates calcium mobilization and regulates intracellular NAD levels, and is implicated having role in various physiological functions (Funaro *et al.,* J Immunology 145:2390-6, 1990; Terhorst *et al.,* Cell 771-80, 1981; Guse *et al.,* Nature 398:70-3, 1999; Adriouch *et al.,* 14:1284-92, 2012; Chiarugi *et al.,* Nature Reviews 12:741-52, 2012; Wei *et al.,* WJBC 5:58-67, 2014)

Acute myeloid leukemia (AML) is a heterogeneous hematologic disorder characterized by clonal expansion of myeloid blasts in bone marrow, peripheral blood and other tissues. Despite recent progress, current treatment of AML remains unsatisfactory with a 5-year relapse-free survival rate lower than 30%.

Therefore, there remains a need for effective treatments for AML.

SUMMARY OF THE INVENTION

One embodiment of the invention is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody for a time sufficient to treat AML.

One embodiment of the invention is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody that competes for binding to CD38 with an antibody comprising a heavy chain variable region (VH) of SEQ ID NO: 4 and a light chain variable region (VL) of SEQ ID NO: 5 for a time sufficient to treat AML.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows daratumumab-induced apoptosis in the absence of crosslinking in NB-4 AML cell line. PI: propidium iodide.

Figure 1B shows daratumumab-induced apoptosis in the presence of crosslinking in NB-4 AML cell line. PI: propidium iodide.

Figure 2A shows the efficacy of daratumumab in patient-derived xenograft (PDX) AML 3406 model as measured by reduction in percentage (%) leukemic $CD45^+CD33^+$ cells in bone marrow (BM), spleen (SPL) and peripheral blood (PB). Ctrl: no treatment; IgG1: isotype control; Dara: daratumumab. p values are indicated in the Figure (isotype control vs. daratumumab).

Figure 2B shows the efficacy of daratumumab in patient-derived xenograft (PDX) AML 7577 model as measured by reduction in percentage (%) leukemic $CD45^+CD33^+$ cells in bone marrow (BM), spleen (SPL) and peripheral blood (PB). Ctrl: no treatment; IgG1: isotype control; Dara: daratumumab. ns: not significant. ***$p<0.001$

Figure 2C shows the efficacy of daratumumab in patient-derived xenograft (PDX) AML 8096 model, assessed by reduction in percentage (%) leukemic $CD45^+CD33^+$ cells in bone marrow (BM), spleen (SPL) and peripheral blood (PB). Ctrl: no treatment; IgG1: isotype control; Dara: daratumumab. ns: not significant. *$p<0.05$

Figure 3A shows the efficacy of daratumumab in patient-derived xenograft (PDX) AML 3406 model, assessed by reduction in total leukemic burden in bone marrow (number of $CD45^+CD33^+$ cells per four bones). Ctrl: no treatment; IgG1: isotype control; Dara: daratumumab. There was no significant difference ($p>0.01$) in bone marrow leukemic burden between Ctrl and Dara. p value between isotype control vs daratumumab treatment groups shown.

Figure 3B shows the efficacy of daratumumab in patient-derived xenograft (PDX) AML 3406 model, assessed by reduction in total leukemic burden in spleen (number of $CD45^+CD33^+$ cells per spleen). Ctrl: no treatment; IgG1: isotype control; Dara: daratumumab. p value between isotype control vs daratumumab treatment groups shown.

Figure 3C shows the efficacy of daratumumab in patient-derived xenograft (PDX) AML 3406 model, assessed by reduction in total leukemic burden in peripheral blood (number of $CD45^+CD33^+$ cells per µl blood). Ctrl: no treatment; IgG1: isotype control; Dara: daratumumab. p value between isotype control vs daratumumab treatment groups is indicated.

Figure 4A shows daratumumab-induced downregulation of surface CD38 expression in patient-derived xenograft (PDX) AML 3406 model in bone marrow (BM), spleen (SPL) and peripheral blood (PB) after 5 weeks of treatment with daratumumab. Ctrl: no treatment; IgG1: isotype control; Dara: daratumumab. p values as indicated in the Figure for isotype control vs. daratumumab.

Figure 4B shows daratumumab-induced reduction in the percentage of CD38-positive leukemia blasts in patient-derived xenograft (PDX) AML 3406 model in bone marrow (BM), spleen (SPL) and peripheral blood (PB) after 5 weeks of treatment with daratumumab. Ctrl: no treatment; IgG1: isotype control; Dara: daratumumab. p values are indicated in between isotype control vs. daratumumab treatment groups.

Figure 5A shows the efficacy of daratumumab (dara) alone or in combination with dacogen (DAC) or cytrabine and doxorubicin (chemo) in reducing leukemia burden in patient-derived xenograft (PDX) 3406 model in bone marrow. Leukemia burden was assessed as % of $CD45^+CD33^+$ cells. Ctrl: isotype control. *p<0.05; **p<0.01; ***p<.001. ns: not significant.

Figure 5B shows the efficacy of daratumumab (dara) alone or in combination with dacogen (DAC) or cytrabine and doxorubicin (chemo) in reducing leukemia burden in patient-derived xenograft (PDX) 3406 model in spleen. Leukemia burden was assessed as % of $CD45^+CD33^+$ cells. Ctrl: isotype control. *p<0.05; **p<0.01; ***p<0.001. ns: not significant.

Figure 5C shows the efficacy of daratumumab (dara) alone or in combination with dacogen (DAC) or cytrabine and doxorubicin (chemo) in reducing leukemia burden in patient-derived xenograft (PDX) model in peripheral blood. Leukemia burden was assessed as % of $CD45^+CD33^+$ cells. Ctrl: isotype control. *p<0.05; **p<0.01; ***p<0.001. ns: not significant.

Figure 6A shows the effect of daratumumab (dara) alone or in combination with dacogen (DAC) or cytrabine and doxorubicin (chemo) on CD38 expression on $CD45^+CD33^+$ AML bone marrow blasts in patient derived xenograft (PDX) 3406 model. Leukemia burden was assessed as % of $CD45^+CD33^+$ cells. Ctrl: isotype control. *p<0.05; **p<0.01; ***p<0.001. ns: not significant. MFI: mean fluorescent intensity.

Figure 6B shows the effect of daratumumab (dara) alone or in combination with dacogen (DAC) or cytrabine and doxorubicin (chemo) on CD38 expression on $CD45^+CD33^+$ AML spleen blasts in patient derived xenograft (PDX) 3406 model. Leukemia burden was assessed as % of $CD45^+CD33^+$ cells. Ctrl: isotype control. *p<0.05; **p<0.01; ***p<0.001. ns: not significant.

Figure 6C shows the effect of daratumumab (dara) alone or in combination with dacogen (DAC) or cytrabine and doxorubicin (chemo) on CD38 expression on $CD45^+CD33^+$ AML peripheral blood blasts in patient derived xenograft (PDX) 3406 model. Leukemia burden was assessed as % of $CD45^+CD33^+$ cells. Ctrl: isotype control. *p<0.05; **p<0.01; ***p<0.001. ns: not significant.

DETAILED DESCRIPTION OF THE INVENTION

"CD38" refers to the human CD38 protein (synonyms: ADP-ribosyl cyclase 1, cADPr hydrolase 1, cyclic ADP-ribose hydrolase 1). Human CD38 has an amino acid sequence shown in SEQ ID NO: 1

"Antibodies" as used herein is meant in a broad sense and includes immunoglobulin molecules including monoclonal antibodies including murine, human, human-adapted, humanized and chimeric monoclonal antibodies, antibody fragments, bispecific or multispecific antibodies, dimeric, tetrameric or multimeric antibodies, and single chain antibodies.

Immunoglobulins may be assigned to five major classes, namely IgA, IgD, IgE, IgG and IgM, depending on the heavy chain constant domain amino acid sequence. IgA and IgG are further sub-classified as the isotypes IgA_1, IgA_2, IgG_1, IgG_2, IgG_3 and IgG_4. Antibody light chains of any vertebrate species may be assigned to one of two clearly distinct types, namely kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

"Antibody fragments" refers to a portion of an immunoglobulin molecule that retains the heavy chain and/or the light chain antigen binding site, such as heavy chain complementarity determining regions (HCDR) 1, 2 and 3, light chain complementarity determining regions (LCDR) 1, 2 and 3, a heavy chain variable region (VH), or a light chain variable region (VL). Antibody fragments include a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; a $F(ab)_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and CH1 domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a domain antibody (dAb) fragment (Ward *et al* (1989) *Nature* 341:544- 546), which consists of a VH domain. VH and VL domains may be engineered and linked together via a synthetic linker to form various types of single chain antibody designs where the VH/VL domains pair intramolecularly, or intermolecularly in those cases when the VH and VL domains are expressed by separate single chain antibody constructs, to form a monovalent antigen binding site, such as single chain Fv (scFv) or diabody; described for example in PCT Intl. Publ. Nos. WO1998/44001, WO1988/01649, WO1994/13804, and WO1992/01047. These antibody fragments are obtained using well known techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are full length antibodies.

The phrase "isolated antibody" refers to an antibody or antibody fragment that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody specifically binding CD38 is substantially free of antibodies that specifically bind antigens other than human CD38). An isolated antibody that specifically binds CD38, however, may have cross-reactivity to other antigens, such as orthologs of human CD38, such as *Macaca fascicularis* (cynomolgus) CD38. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

An antibody variable region consists of a "framework" region interrupted by three "antigen binding sites". The antigen binding sites are defined using various terms: Complementarity Determining Regions (CDRs), three in the VH (HCDR1, HCDR2, HCDR3) and three in the VL (LCDR1, LCDR2, LCDR3) are based on sequence variability (Wu and Kabat J Exp Med 132:211-50, 1970; Kabat et al Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991), "Hypervariable regions", "HVR", or "HV", three in the VH (H1, H2, H3) and three in the VL (L1, L2, L3) refer to the regions of an antibody variable domains which are hypervariable in structure as defined by Chothia and Lesk (Chothia and Lesk Mol Biol 196:901-17, 1987). Other terms include "IMGT-CDRs" (Lefranc *et al.*, Dev Comparat Immunol 27:55-77, 2003) and "Specificity Determining Residue Usage" (SDRU) (Almagro, Mol Recognit 17:132-43, 2004). The International ImMunoGeneTics (IMGT) database (http://www_imgt_org) provides a standardized numbering and definition of antigen-binding sites. The correspondence between CDRs, HVs and IMGT delineations is described in Lefranc *et al.*, Dev Comparat Immunol 27:55-77, 2003.

"Chothia residues" as used herein are the antibody VL and VH residues numbered according to Al-Lazikani (Al-Lazikani *et al.*, J Mol Biol 273:927-48, 1997).

"Framework" or "framework sequences" are the remaining sequences of a variable region other than those defined to be antigen binding sites. Because the antigen binding sites may be defined by various terms as described above, the exact amino acid sequence of a framework depends on how the antigen-binding site was defined.

"Humanized antibody" refers to an antibody in which the antigen binding sites are derived from non-human species and the variable region frameworks are derived from human immunoglobulin sequences. Humanized antibodies may include substitutions in the framework regions so that the framework may not be an exact copy of expressed human immunoglobulin or germline gene sequences.

"Human-adapted" antibodies or "human framework adapted (HFA)" antibodies refers to humanized antibodies adapted according to methods described in U.S. Pat. Publ.

No. US2009/0118127. Human-adapted antibodies are humanized by selecting the acceptor human frameworks based on the maximum CDR and FR similarities, length compatibilities and sequence similarities of CDR1 and CDR2 loops and a portion of light chain CDR3 loops.

"Human antibody" refers to an antibody having heavy and light chain variable regions in which both the framework and the antigen binding sites are derived from sequences of human origin. If the antibody contains a constant region, the constant region also is derived from sequences of human origin.

A human antibody comprises heavy or light chain variable regions that are "derived from" sequences of human origin wherein the variable regions of the antibody are obtained from a system that uses human germline immunoglobulin or rearranged immunoglobulin genes. Such systems include human immunoglobulin gene libraries displayed on phage, and transgenic non-human animals such as mice carrying human immunoglobulin loci as described herein. A "human antibody" may contain amino acid differences when compared to the human germline or rearranged immunoglobulin sequences due to for example naturally occurring somatic mutations or intentional introduction of substitutions in the framework or antigen binding sites. Typically, a human antibody is at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical in amino acid sequence to an amino acid sequence encoded by a human germline or rearranged immunoglobulin gene. In some cases, "human antibody" may contain consensus framework sequences derived from human framework sequence analyses, for example as described in Knappik *et al.*, J Mol Biol 296:57-86, 2000), or synthetic HCDR3 incorporated into human immunoglobulin gene libraries displayed on phage, for example as described in Shi *et al.*, J Mol Biol 397:385-96, 2010 and Intl. Pat. Publ. No. WO2009/085462). Antibodies in which antigen binding sites are derived from a non-human species are not included in the definition of human antibody.

Isolated humanized antibodies may be synthetic. Human antibodies may be generated using systems such as phage display incorporating synthetic CDRs and/or synthetic frameworks, or can be subjected to *in vitro* mutagenesis to improve antibody properties.

"Recombinant antibody" as used herein includes all antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from an animal, for example a mouse or a rat, that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom (described further below),

antibodies isolated from a host cell transformed to express the antibody, antibodies isolated from a recombinant, combinatorial antibody library, and antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences, or antibodies that are generated *in vitro* using for example Fab arm exchange to generate bispecific antibodies.

"Monoclonal antibody" as used herein refers to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope, or in a case of a bispecific monoclonal antibody, a dual binding specificity to two distinct epitopes.

"Epitope" as used herein means a portion of an antigen to which an antibody specifically binds. Epitopes usually consist of chemically active (such as polar, non-polar or hydrophobic) surface groupings of moieties such as amino acids or polysaccharide side chains and can have specific three-dimensional structural characteristics, as well as specific charge characteristics. Epitope may be composed of contiguous and/or discontiguous amino acids that form a conformational spatial unit. For a discontiguous epitope, amino acids from differing portions of the linear sequence of the antigen come in close proximity in 3-dimensional space through the folding of the protein molecule.

"Variant" as used herein refers to a polypeptide or a polynucleotide that differs from a reference polypeptide or a reference polynucleotide by one or more modifications for example, substitutions, insertions or deletions.

"Synergy", "synergism" or "synergistic" mean more than the expected additive effect of a combination.

The term "in combination with" as used herein means that two or more therapeutics can be administered to a subject together in a mixture, concurrently as single agents or sequentially as single agents in any order.

"Treat" or "treatment" refers to therapeutic treatment wherein the object is to slow down (lessen) an undesired physiological change or disease, such as the development, expansion or spread of tumor or tumor cells, or to provide a beneficial or desired clinical outcome during treatment. Beneficial or desired clinical outcomes include alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" may also mean prolonging survival as compared to expected survival if a subject was not receiving treatment. Those in need of treatment include those subjects

already with the undesired physiological change or disease as well as those subjects prone to have the physiological change or disease.

"Inhibits growth" (e.g. referring to cells, such as tumor cells) refers to a measurable decrease in the cell growth *in vitro* or *in vivo* when contacted with a therapeutic or a combination of therapeutics or drugs when compared to the growth of the same cells grown in appropriate control conditions well known to the skilled in the art. Inhibition of growth of a cell *in vitro* or *in vivo* may be at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 99%, or 100%. Inhibition of cell growth may occur by a variety of mechanisms, for example by antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), complement dependent cytotoxicity (CDC), apoptosis, necrosis, inhibition of CD38 enzymatic activity, or by inhibition of cell proliferation.

A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of a therapeutic or a combination of therapeutics to elicit a desired response in the individual. Exemplary indicators of an effective therapeutic or combination of therapeutics include, for example, improved well-being of the patient, reduction of a tumor burden, arrested or slowed growth of a tumor, and/or absence of metastasis of cancer cells to other locations in the body.

One embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody for a time sufficient to treat AML.

Another embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody that competes for binding to CD38 with an antibody comprising a heavy chain variable region (VH) of SEQ ID NO: 4 and a light chain variable region (VL) of SEQ ID NO: 5 for a time sufficient to treat AML.

Another embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody that binds to the region SKRNIQFSCKNIYR (SEQ

ID NO: 2) and the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1) for a time sufficient to treat AML.

An anti-CD38 antibody binds to the region SKRNIQFSCKNIYR (SEQ ID NO: 2) and the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1) when the antibody binds at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 residues within SEQ ID NO: 2 and SEQ ID NO: 3. In some embodiments disclosed herein, including the numbered embodiments listed below, the anti-CD38 antibody binds at least one amino acid in the region SKRNIQFSCKNIYR (SEQ ID NO: 2) and at least one amino acid in the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1). In some embodiments disclosed herein, including in the numbered embodiments listed below, the anti-CD38 antibody binds at least two amino acids in the region SKRNIQFSCKNIYR (SEQ ID NO: 2) and at least two amino acids in the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1). In some embodiments disclosed herein, including in the numbered embodiments listed below, the anti-CD38 antibody binds at least three amino acids in the region SKRNIQFSCKNIYR (SEQ ID NO: 2) and at least three amino acids in the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1). In some embodiments disclosed herein, including in the numbered embodiments listed below, the anti-CD38 antibody binds at least residues KRN in the region SKRNIQFSCKNIYR (SEQ ID NO: 2) and at least residues VQLT (SEQ ID NO: 14) in the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1).

An exemplary antibody that binds to the region SKRNIQFSCKNIYR (SEQ ID NO: 2) and the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1) or minimally to residues KRN and VQLT (SEQ ID NO: 14) as shown above is daratumumab (see Intl. Pat. Publ. No. WO2006/0998647). Daratumumab comprises the VH and the VL amino acid sequences shown in SEQ ID NO: 4 and 5, respectively, heavy chain CDRs HCDR1, HCDR2 and HCDR3 of SEQ ID NOs: 6, 7 and 8, respectively, and light chain CDRs LCDR1, LCDR2 and LCDR3 of SEQ ID NOs: 9, 10 and 11, respectively, and is of IgG1/κ subtype. Daratumumab heavy chain amino acid sequence is shown in SEQ ID NO: 12 and light chain amino acid sequence shown in SEQ ID NO: 13.

Another embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody comprising a heavy chain variable region (VH) and

a light chain variable region (VL) of SEQ ID NOs: 4 and 5, respectively, for a time sufficient to treat AML.

Another embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody comprising heavy chain CDRs HCDR1, HCDR2 and HCDR3 of SEQ ID NOs: 6, 7 and 8, respectively, and light chain CDRs LCDR1, LCDR2 and LCDR3 of SEQ ID NOs: 9, 10 and 11, respectively, for a time sufficient to treat AML.

SEQ ID NO: 1
MANCEFSPVSGDKPCCRLSRRAQLCLGVSILVLILVVVLAVVVPRWRQQWSGPGT
TKRFPETVLARCVKYTEIHPEMRHVDCQSVWDAFKGAFISKHPCNITEEDYQPLM
KLGTQTVPCNKILLWSRIKDLAHQFTQVQRDMFTLEDTLLGYLADDLTWCGEFN
TSKINYQSCPDWRKDCSNNPVSVFWKTVSRRFAEAACDVVHVMLNGSRSKIFDK
NSTFGSVEVHNLQPEKVQTLEAWVIHGGREDSRDLCQDPTIKELESIISKRNIQFSC
KNIYRPDKFLQCVKNPEDSSCTSEI

SEQ ID NO: 2
SKRNIQFSCKNIYR

SEQ ID NO: 3
EKVQTLEAWVIHGG

SEQ ID NO: 4
EVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMSWVRQAPGKGLEWVSA
ISGSGGGTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYFCAKDK
ILWFGEPVFDYWGQGTLVTVSS

SEQ ID NO: 5
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYD
ASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPTFGQ
GTKVEIK

SEQ ID NO: 6
SFAMS

SEQ ID NO: 7
AISGSGGGTYYADSVKG

SEQ ID NO: 8
DKILWFGEPVFDY

SEQ ID NO: 9
RASQSVSSYLA

SEQ ID NO: 10
DASNRAT

SEQ ID NO: 11
QQRSNWPPTF

SEQ ID NO: 12
EVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMSWVRQAPGKGLEWVSAISGSG
GGTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYFCAKDKILWFGEPVF
DYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN
SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLSPGK

SEQ ID NO: 13
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRAT
GIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPTFGQGTKVEIKRTVAAP
SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 14

VQLT

Antibodies may be evaluated for their competition with daratumumab having VH of SEQ ID NO: 4 and VL of SEQ ID NO: 5 for binding to CD38 using well known *in vitro* methods. In an exemplary method, CHO cells recombinantly expressing CD38 may be incubated with unlabeled daratumumab for 15 min at 4°C, followed by incubation with an excess of fluorescently labeled test antibody for 45 min at 4°C. After washing in PBS/BSA, fluorescence may be measured by flow cytometry using standard methods. In another exemplary method, extracellular portion of human CD38 may be coated on the surface of an ELISA plate. Excess of unlabelled daratumumab may be added for about 15 minutes and subsequently biotinylated test antibodies may be added. After washes in PBS/Tween, binding of the test biotinylated antibody may be detected using horseradish peroxidase (HRP)-conjugated streptavidine and the signal detected using standard methods. It is readily apparent that in the competition assays, daratumumab may be labelled and the test antibody unlabeled. The test antibody competes with daratumumab when daratumumab inhibits binding of the test antibody, or the test antibody inhibits binding of daratumumab by 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90% , 95% or 100%. The epitope of the test antibody may further be defined for example by peptide mapping or hydrogen/deuterium protection assays using known methods, or by crystal structure determination.

Antibodies binding to the same region on CD38 as daratumumab may be generated for example by immunizing mice with peptides having the amino acid sequences shown in SEQ ID NOs: 2 and 3 using standard methods and as described herein. Antibodies may be further evaluated for example by assaying competition between daratumumab and a test antibody for binding to CD38 using well known *in vitro* methods and as described herein.

Other exempary anti-CD38 antibodies that may be used in any embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, are:

mAb003 comprising the VH and VL sequences of SEQ ID NOs: 15 and 16, respectively and described in U.S. Pat. No. 7,829,693. The VH and the VL of mAb003 may be expressed as IgG1/κ.

SEQ ID NO: 15

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPGQGLEWMGRVIPF
LGIANSAQKFQGRVTITADKSTSTAY
MDLSSLRSEDTAVYYCARDDIAALGPFDYWGQGTLVTVSSAS

SEQ ID NO: 16

DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPRTFGQGTKVEIK;

mAb024 comprising the VH and VL sequences of SEQ ID NOs: 17 and 18, respectively, described in U.S. Pat. No. 7,829,693. The VH and the VL of mAb024 may be expressed as IgG1/κ.

SEQ ID NO: 17

EVQLVQSGAEVKKPGESLKISCKGSGYSFSNYWIGWVRQMPGKGLEWMGIIYPH
DSDARYSPSFQGQVTFSADKSISTAYLQWSSLKASDTAMYYCARHVGWGSRYW
YFDLWGRGTLVTVSS

SEQ ID NO: 18

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRAT
GIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPTFGQGTKVEIK;

MOR-202 (MOR-03087) comprising the VH and VL sequences of SEQ ID NOs: 19 and 20, respectively, described in US. Pat. No. 8,088,896. The VH and the VL of MOR-202 may be expressed as IgG1/κ.

SEQ ID NO: 19

QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYMNWVRQAPGKGLEWVSGISGD
PSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDLPLVYTGFA
YWGQGTLVTVSS

SEQ ID NO: 20

DIELTQPPSVSVAPGQTARISCSGDNLRHYYVYWYQQKPGQAPVLVIYGDSKRPS
GIPERFSGSNSGNTATLTISGTQAEDEADYYCQTYTGGASLVFGGGTKLTVLGQ;

Isatuximab; comprising the VH and VL sequences of SEQ ID NOs: 21 and 22, respectively, described in U.S. Pat. No. 8,153,765. The VH and the VL of Isatuximab may be expressed as IgG1/κ.

SEQ ID NO 21:
QVQLVQSGAEVAKPGTSVKLSCKASGYTFTDYWMQWVKQRPGQGLEWIGT
IYPGDGDTGYAQKFQGKATLTADKSSKTVYMHLSSLASEDSAVYYCARGD
YYGSNSLDYWGQGTSVTVSS

SEQ ID NO: 22:
DIVMTQSHLSMSTSLGDPVSITCKASQDVSTVVAWYQQKPGQSPRRLIYS
ASYRYIGVPDRFTGSGAGTDFTFTISSVQAEDLAVYYCQQHYSPPYTFGG
GTKLEIK.

Other exemplary anti-CD38 antibodies that may be used in the methods of the invention include those described in Int. Pat. Publ. No. WO05/103083, Intl. Pat. Publ. No. WO06/125640, Intl. Pat. Publ. No. WO07/042309, Intl. Pat. Publ. No. WO08/047242 or Intl. Pat. Publ. No. WO14/178820.

Another embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody comprising a heavy chain variable region (VH) and a light chain variable region (VL) of SEQ ID NOs: 15 and 16, respectively, for a time sufficient to treat AML.

Another embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody comprising a heavy chain variable region (VH) and a light chain variable region (VL) of SEQ ID NOs: 17 and 18, respectively, for a time sufficient to treat AML.

Another embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody comprising a heavy chain variable region (VH) and a light chain variable region (VL) of SEQ ID NOs: 19 and 20, respectively, for a time sufficient to treat AML.

Another embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody comprising a heavy chain variable region (VH) and a light chain variable region (VL) of SEQ ID NOs: 21 and 22, respectively, for a time sufficient to treat AML.

The Fc portion of the antibody may mediate antibody effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) or complement dependent cytotoxicity (CDC). Such function may be mediated by binding of an Fc effector domain(s) to an Fc receptor on an immune cell with phagocytic or lytic activity or by binding of an Fc effector domain(s) to components of the complement system. Typically, the effect(s) mediated by the Fc-binding cells or complement components result in inhibition and/or depletion of target cells, for example CD38-expressing cells. Human IgG isotypes IgG1, IgG2, IgG3 and IgG4 exhibit differential capacity for effector functions. ADCC may be mediated by IgG1 and IgG3, ADCP may be mediated by IgG1, IgG2, IgG3 and IgG4, and CDC may be mediated by IgG1 and IgG3.

In the methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody is of IgG1, IgG2, IgG3 or IgG4 isotype.

In the methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody induces killing of AML cells that express CD38 by apoptosis.

The anti-CD38 antibodies used in the methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, may induce killing of AML cells by apoptosis. Methods for evaluating apoptosis are well known, and include for example annexin IV staining using standard methods. The anti-CD38 antibodies used in the methods of the invention may induce apoptosis in about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% of cells.

In the methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 induces killing of AML cells that express CD38 by ADCC.

In the methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 induces killing of AML cells that express CD38 by CDC.

In the methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody induces killing of AML cells that express CD38 by ADCP.

In the methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody induces killing of AML cells that express CD38 by ADCC and CDC.

"Antibody-dependent cellular cytotoxicity", "antibody-dependent cell-mediated cytotoxicity" or "ADCC" is a mechanism for inducing cell death that depends upon the interaction of antibody-coated target cells with effector cells possessing lytic activity, such as natural killer cells, monocytes, macrophages and neutrophils via Fc gamma receptors (FcγR) expressed on effector cells. For example, NK cells express FcγRIIIa, whereas monocytes express FcγRI, FcγRII and FcvRIIIa. Death of the antibody-coated target cell, such as CD38-expressing cells, occurs as a result of effector cell activity through the secretion of membrane pore-forming proteins and proteases. To assess ADCC activity of an anti-CD38 antibody, the antibody may be added to CD38-expressing cells in combination with immune effector cells, which may be activated by the antigen antibody complexes resulting in cytolysis of the target cell. Cytolysis is generally detected by the release of label (e.g. radioactive substrates, fluorescent dyes or natural intracellular proteins) from the lysed cells. Exemplary effector cells for such assays include peripheral blood mononuclear cells (PBMC) and NK cells. Exemplary target cells include Daudi cells (ATCC® CCL-213™) or B cell leukemia or lymphoma tumor cells expressing CD38. In an exemplary assay, target cells are labeled with 20 μCi of ^{51}Cr for 2 hours and washed extensively. Cell concentration of the target cells may be adjusted to 1×10^{6} cells/ml, and anti-CD38 antibodies at various concentrations are added. Assays are started by adding Daudi cells at an effector:target cell ratio of 40:1. After incubation for 3 hr at 37°C assays are stopped by centrifugation, and ^{51}Cr release from lysed cells are measured in a scintillation counter. Percentage of cellular cytotoxicity may be calculated as % maximal lysis which may be induced by adding 3% perchloric acid to target cells. Anti-CD38 antibodies used in the methods of the invention may induce ADCC by about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% of control (cell lysis induced by 3% perchloric acid).

"Antibody-dependent cellular phagocytosis" ("ADCP") refers to a mechanism of elimination of antibody-coated target cells by internalization by phagocytic cells, such as macrophages or dendritic cells. ADCP may be evaluated by using monocyte-derived macrophages as effector cells and Daudi cells (ATCC® CCL-213™) or B cell leukemia or lymphoma tumor cells expressing CD38 as target cells engineered to express GFP or other labeled molecule. Effctor:target cell ratio may be for example 4:1. Effector cells may be incubated with target cells for 4 hours with or without anti-CD38 antibody. After incubation, cells may be detached using accutase. Macrophages may be identified with anti-CD11b and anti-CD14 antibodies coupled to a fluorescent label, and percent phagocytosis may be determined based on % GFP fluorescent in the $CD11^{+}CD14^{+}$ macrophages using standard methods. Anti-CD38 antibodies used in the methods of the invention may induce ADCP by about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% .

"Complement-dependent cytotoxicity", or "CDC", refers to a mechanism for inducing cell death in which an Fc effector domain of a target-bound antibody binds and activates complement component C1q which in turn activates the complement cascade leading to target cell death. Activation of complement may also result in deposition of complement components on the target cell surface that facilitate ADCC by binding complement receptors (e.g., CR3) on leukocytes. CDC of CD38-expressing cells may be measured for example by plating Daudi cells at 1×10^{5} cells/well (50 µl/well) in RPMI-B (RPMI supplemented with 1% BSA), adding 50 µl anti-CD38 antibodies to the wells at final concentration between 0-100 µg/ml, incubating the reaction for 15 min at room temperature, adding 11 µl of pooled human serum to the wells, and incubating the reaction for 45 min at 37° C. Percentage (%) lysed cells may be detected as % propidium iodide stained cells in FACS assay using standard methods. Anti-CD38 antibodies used in the methods of the invention may induce CDC by about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% .

The ability of monoclonal antibodies to induce ADCC may be enhanced by engineering their oligosaccharide component. Human IgG1 or IgG3 are N-glycosylated at Asn297 with the majority of the glycans in the well-known biantennary G0, G0F, G1, G1F, G2 or G2F forms. Antibodies produced by non-engineered CHO cells typically have a glycan fucose content of about at least 85%. The removal of the core fucose from the biantennary complex-type oligosaccharides attached to the Fc regions enhances the ADCC of antibodies via improved FcγRIIIa binding without altering antigen binding or CDC activity. Such mAbs may be achieved using different methods reported to lead to the

successful expression of relatively high defucosylated antibodies bearing the biantennary complex-type of Fc oligosaccharides such as control of culture osmolality (Konno *et al.*, Cytotechnology 64:249-65, 2012), application of a variant CHO line Lec13 as the host cell line (Shields *et al.*, J Biol Chem 277:26733-26740, 2002), application of a variant CHO line EB66 as the host cell line (Olivier *et al.*, MAbs ;2(4), 2010; Epub ahead of print; PMID:20562582), application of a rat hybridoma cell line YB2/0 as the host cell line (Shinkawa *et al.*, J Biol Chem 278:3466-3473, 2003), introduction of small interfering RNA specifically against the α 1,6-fucosyltrasferase (*FUT8)* gene (Mori *et al.*, Biotechnol Bioeng88:901-908, 2004), or coexpression of β-1,4-*N*-acetylglucosaminyltransferase III and Golgi α-mannosidase II or a potent alpha-mannosidase I inhibitor, kifunensine (Ferrara *et al.*, J Biol Chem281:5032-5036, 2006, Ferrara *et al.*, Biotechnol Bioeng 93:851-861, 2006; Xhou *et al.*, Biotechnol Bioeng 99:652-65, 2008). ADCC elicited by anti-CD38 antibodies used in the methods of the invention, and in some embodiments of each and every one of the numbered embodiments listed below, may also be enhanced by certain substitutions in the antibody Fc. Exemplary substitutions are for example substitutions at amino acid positions 256, 290, 298, 312, 356, 330, 333, 334, 360, 378 or 430 (residue numbering according to the EU index) as described in U.S. Pat. No. 6,737,056.

In some methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibodies comprise a substitution in the antibody Fc.

In some methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibodies comprise a substitution in the antibody Fc at amino acid positions 256, 290, 298, 312, 356, 330, 333, 334, 360, 378 or 430 (residue numbering according to the EU index).

In some methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody has a biantennary glycan structure with fucose content of about between 0% to about 15%, for example 15%, 14%, 13%, 12%, 11% 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1% or 0%.

In some methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody has a biantennary glycan structure with fucose content of about 50%, 40%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 14%, 13%, 12%, 11% 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1% or 0%

Substitutions in the Fc and reduced fucose content may enhance the ADCC activity of the anti-CD38 antibody.

"Fucose content" means the amount of the fucose monosaccharide within the sugar chain at Asn297. The relative amount of fucose is the percentage of fucose-containing structures related to all glycostructures. These may be characterized and quantified by multiple methods, for example: 1) using MALDI-TOF of N-glycosidase F treated sample (e.g. complex, hybrid and oligo- and high-mannose structures) as described in Intl. Pat. Publ. No. WO2008/077546; 2) by enzymatic release of the Asn297 glycans with subsequent derivatization and detection/ quantitation by HPLC (UPLC) with fluorescence detection and/or HPLC-MS (UPLC-MS); 3) intact protein analysis of the native or reduced mAb, with or without treatment of the Asn297 glycans with Endo S or other enzyme that cleaves between the first and the second GlcNAc monosaccharides, leaving the fucose attached to the first GlcNAc; 4) digestion of the mAb to constituent peptides by enzymatic digestion (e.g., trypsin or endopeptidase Lys-C), and subsequent separation, detection and quantitation by HPLC-MS (UPLC-MS) or 5) separation of the mAb oligosaccharides from the mAb protein by specific enzymatic deglycosylation with PNGase F at Asn 297. The oligosaccharides released can be labeled with a fluorophore, separated and identified by various complementary techniques which allow: fine characterization of the glycan structures by matrix-assisted laser desorption ionization (MALDI) mass spectrometry by comparison of the experimental masses with the theoretical masses, determination of the degree of sialylation by ion exchange HPLC (GlycoSep C), separation and quantification of the oligosacharride forms according to hydrophilicity criteria by normal-phase HPLC (GlycoSep N), and separation and quantification of the oligosaccharides by high performance capillary electrophoresis-laser induced fluorescence (HPCE-LIF).

"Low fucose" or "low fucose content" as used in the application refers to antibodies with fucose content of about 0% - 15%.

"Normal fucose" or 'normal fucose content" as used herein refers to antibodies with fucose content of about over 50%, typically about over 60%, 70%, 80% or over 85%.

The anti-CD38 antibodies used in the methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, may induce killing of AML cells by modulation of CD38 enzymatic activity. CD38 is a multifunctional ectoenzme with ADP-ribosyl cyclase activity catalyzing the formation of cyclic ADP-ribose (cADPR) and ADPR from NAD^+. CD38 also catalyzes the exchange of the nicotinamide group of $NADP^+$ with nicotinic acid under acidic conditions, to yield $NAADP^+$ (nicotinic acid-adenine dinucleotide phosphate). Modulation of the enzymatic activity of human CD38 with anti-CD38 antibodies used in the methods of the invention

may be measured in an assay described in Graeff *et al.*, J. Biol. Chem. 269, 30260-30267 (1994). For example, substrate NGD^+ may be incubated with CD38, and the modulation of the production of cyclic GDP-ribose (cGDPR) may be monitored spectrophotometrically at excitation at 340 nM and emission at 410 nM at different time points after addition of the antibody at various concentrations. Inhibition of the synthesis of cADPR can be determined according to the HPLC method described in Munshi *et al.*, J. Biol. Chem. 275, 21566-21571 (2000). The anti-CD38 antibodies used in the methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, may inhibit CD38 enzymatic activity by at least about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% .

In some methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody comprises the heavy chain complementarity determining regions (HCDR) 1 (HCDR1), 2 (HCDR2) and 3 (HCDR3) sequences of SEQ ID NOs: 6, 7 and 8, respectively.

In some methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody comprises the light chain complementarity determining regions (LCDR) 1 (LCDR1), 2 (LCDR2) and 3 (LCDR3) sequences of SEQ ID NOs: 9, 10 and 11, respectively.

In some methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody comprises the heavy chain complementarity determining regions (HCDR) 1 (HCDR1), 2 (HCDR2) and 3 (HCDR3) sequences of SEQ ID NOs: 6, 7 and 8, respectively, and the light chain complementarity determining regions (LCDR) 1 (LCDR1), 2 (LCDR2) and 3 (LCDR3) sequences of SEQ ID NOs: 9, 10 and 11, respectively.

In some methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody comprises the heavy chain variable region (VH) of SEQ ID NO: 4 and the light chain variable region (VL) of SEQ ID NO: 5.

In some methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody comprises a heavy chain of SEQ ID NO: 12 and a light chain of SEQ ID NO: 13.

In some methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody comprises a heavy chain comprising an amino acid sequence that is 95%, 96%, 97%, 98%

or 99% identical to that of SEQ ID NO: 12 and a light chain comprising an amino acid sequence that is 95%, 96%, 97%, 98% or 99% identical to that of SEQ ID NO: 13.

Antibodies that are substantially identical to the antibody comprising the heavy chain of SEQ ID NO: 12 and the light chain of SEQ ID NO: 13 may be used in the methods of the invention. "Substantially identical" as used herein means that the two antibody heavy chain or light chain amino acid sequences being compared are identical or have "insubstantial differences". Insubstantial differences are substitutions of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids in an antibody heavy chain or light chain that do not adversely affect antibody properties. Percent identity can be determined for example by pairwise alignment using the default settings of the AlignX module of Vector NTI v.9.0.0 (Invitrogen, Carlsbad, CA). The protein sequences of the present invention may be used as a query sequence to perform a search against public or patent databases to, for example, identify related sequences. Exemplary programs used to perform such searches are the XBLAST or BLASTP programs (http_//www_ncbi_nlm/nih_gov), or the GenomeQuest™ (GenomeQuest, Westborough, MA) suite using the default settings. Exemplary substitutions that may be made to the anti-CD38 antibodies used in the methods of the invention are for example conservative substitutions with an amino acid having similar charge, hydrophobic, or stereochemical characteristics. Conservative substitutions may also be made to improve antibody properties, for example stability or affinity, or to improve antibody effector functions. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acid substitutions may be made for example to the heavy or the light chain of the anti-CD38 antibody. Furthermore, any native residue in the heavy or light chain may also be substituted with alanine, as has been previously described for alanine scanning mutagenesis (MacLennan *et al.,* Acta Physiol Scand Suppl 643:55-67, 1998; Sasaki *et al.,* Adv Biophys 35:1-24, 1998). Desired amino acid substitutions may be determined by those skilled in the art at the time such substitutions are desired. Amino acid substitutions may be done for example by PCR mutagenesis (U.S. Pat. No. 4,683,195). Libraries of variants may be generated using well known methods, for example using random (NNK) or non-random codons, for example DVK codons, which encode 11 amino acids (Ala, Cys, Asp, Glu, Gly, Lys, Asn, Arg, Ser, Tyr, Trp) and screening the libraries for variants with desired properties. The generated variants may be tested for their binding to CD38 and their ability to induce apoptosis or modulate CD38 enzymatic activity using methods described herein.

In the methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody may bind human

CD38 with a range of affinities (K_D). In one embodiment according to the invention, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody binds to CD38 with a K_D equal to or less than about $1x10^{-8}$ M, for example $5x10^{-9}$ M, $1x10^{-9}$ M, $5x10^{-10}$ M, $1x10^{-10}$ M, $5x10^{-11}$ M, $1x10^{-11}$ M, $5x10^{-12}$ M, $1x10^{-12}$ M, $5x10^{-13}$ M, $1x10^{-13}$ M, $5x10^{-14}$ M, $1x10^{-14}$ M or $5x10^{-15}$M, or any range or value therein, as determined by surface plasmon resonance or the Kinexa method, as practiced by those of skill in the art. One exemplary affinity is equal to or less than $1x10^{-8}$ M. Another exemplary affinity is equal to or less than $1x10^{-9}$ M.

In some embodiments, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody is a bispecific antibody. The VL and/or the VH regions of existing anti-CD38 antibodies or the VL and VH regions identified *de novo* as described herein may be engineered into bispecific full length antibodies. Such bispecific antibodies may be made by modulating the CH3 interactions in antibody Fc to form bispecific antibodies using technologies such as those described in U.S. Pat. No. 7,695,936; Int. Pat. Publ. No. WO04/111233; U.S. Pat. Publ. No. US2010/0015133; U.S. Pat. Publ. No. US2007/0287170; Int. Pat. Publ. No. WO2008/119353; U.S. Pat. Publ. No. US2009/0182127; U.S. Pat. Publ. No. US2010/0286374; U.S. Pat. Publ. No. US2011/0123532; Int. Pat. Publ. No. WO2011/131746; Int. Pat. Publ. No. WO2011/143545; or U.S. Pat. Publ. No. US2012/0149876.

For example, bispecific antibodies of the invention may be generated *in vitro* in a cell-free environment by introducing asymmetrical mutations in the CH3 regions of two monospecific homodimeric antibodies and forming the bispecific heterodimeric antibody from two parent monospecific homodimeric antibodies in reducing conditions to allow disulfide bond isomerization according to methods described in Intl.Pat. Publ. No. WO2011/131746. In the methods, the first monospecific bivalent antibody (e.g., anti-CD38 antibody) and the second monospecific bivalent antibody are engineered to have certain substitutions at the CH3 domain that promote heterodimer stability; the antibodies are incubated together under reducing conditions sufficient to allow the cysteines in the hinge region to undergo disulfide bond isomerization; thereby generating the bispecific antibody by Fab arm exchange. The incubation conditions may optimally be restored to non-reducing. Exemplary reducing agents that may be used are 2- mercaptoethylamine (2-MEA), dithiothreitol (DTT), dithioerythritol (DTE), glutathione, tris(2-carboxyethyl)phosphine (TCEP), L-cysteine and beta-mercaptoethanol, preferably a reducing agent selected from the group consisting of: 2- mercaptoethylamine,

dithiothreitol and tris(2-carboxyethyl)phosphine. For example, incubation for at least 90 min at a temperature of at least 20°C in the presence of at least 25 mM 2-MEA or in the presence of at least 0.5 mM dithiothreitol at a pH of from 5-8, for example at pH of 7.0 or at pH of 7.4 may be used.

Exemplary CH3 mutations that may be used in a first heavy chain and in a second heavy chain of the bispecific antibody are K409R and/or F405L.

Additional bispecific structures into which the VL and/or the VH regions of the antibodies of the invention may be incorporated are for example Dual Variable Domain Immunoglobulins (DVD) (Int. Pat. Publ. No. WO2009/134776), or structures that include various dimerization domains to connect the two antibody arms with different specificity, such as leucine zipper or collagen dimerization domains (Int. Pat. Publ. No. WO2012/022811, U.S. Pat. No. 5,932,448; U.S. Pat. No. 6,833,441). DVDs are full length antibodies comprising the heavy chain having a structure VH1-linker-VH2-CH and the light chain having the structure VL1-linker-VL2-CL; linker being optional.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody is conjugated to a toxin. Conjugation methods and suitable toxins are well known.

AML diagnosis is performed by a physician according to guidelines available, for example according to the World Health Organization (WHO) classification of AML (Brunning et al., World Health Organization Classificaiton of Tumors, 3, pp77-80; eds. Jaffe et al., Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues) and according to guidelines available for example at National Comprehensive Cancer Network (http://_www_nccn.org/_professionals/_physician_gls/_f_guidelines_asp#site). The WHO classification incorporates clinical features, cytogenetics, immunophenotype, morphology and genetics in order to define biologically homogenous subgroups having therapeutic and prognostic relevance, and divides AML to four main subtypes: AML with recurrent genetic abnormalities, AML with multilineage dysplasia, therapy-related AML, and not otherwise categorized AML.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is AML with at least one genetic abnormality.

AML may be associated with a translocation between chromosomes 8 and 21, translocation or inversion in chromosome 16, translocation between chromosomes 15 and 17, or changes in chromosome 11.

Common chromosomal rearrangements associated with AML are translocations t(8; 21)(q22; q22) (AML1/ETO), inv(16)(p13; q22) or t(16; 16)(p13; q22); (CBFβ/MYH11) or t(15; 17)(q22; q12); (PML/RARA). Patients with these favorable chromosomal translocations may be more susceptible to treatment and achieve higher complete remission (CR) rates.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is associated with a translocation between chromosomes 8 and 21, translocation or inversion in chromosome 16, translocation between chromosomes 15 and 17, or changes in chromosome 11.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is associated with a chromosomal abnormality t(8; 21)(q22; q22) (AML1/ETO), inv(16)(p13; q22) or t(16; 16)(p13; q22); (CBFβ/MYH11) or t(15; 17)(q22; q12); (PML/RARA).

Somatic mutations in various genes have been identified as being relevant to AML pathogenesis. These include mutations in fms-related tyrosine kinase 3 (FLT3), nucleophosmin (NPM1), isocitrate dehydrogenase 1(IDH1), isocitrate dehydrogenase 2 (IDH2), DNA (cytosine-5)-methyltransferase 3 (DNMT3A), CCAAT/enhancer binding protein alpha (CEBPA), U2 small nuclear RNA auxiliary factor 1(U2AF1), enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), structural maintenance of chromosomes 1A (SMC1A) and structural maintenance of chromosomes 3 (SMC3) (The Cancer Genome Atlas Research Network; N Engl J Med 368:2059-74, 2013).

Activating mutations in the FLT3 gene have been described in approximately 20-30% of newly diagnosed AML patients. These include FLT3-ITD, internal tandem duplication mutations as a result of duplication and tandem insertion of parts of the juxtamembrane domain of the FLT3 gene (Schnittger *et al.*, Blood 100:59-66, 2002) and D835 mutations in the FLT3 kinase domain. Patients with FLT3-ITD mutations appear to have reduced overall survival (OS) with increased relapse rate (Kottaridis *et al.*, Blood 98: 1752-9, 2001; Yanada *et al.*, Leukemia 19: 1345-9, 2005).

Mutations in IDH1 and IDH2 are present in about 15% of newly diagnosed patients. IDH1 mutations include substitutions R132H, R132X (X being any amino acid) and R100Q/R104V/F108L/R119Q/I130V and IDH2 mutations include substitutions R140Q and R172. IDH1/2 mutations are associated with poorer prognosis, except that $IDH2^{R140Q}$ is associated with somewhat prolonged survival (Molenaar *et al.*, Biochim Biophys Acta 1846: 326-41, 2014). IDH1/2 mutation frequency increases with disease progression (Molenaar et al., Biochim Biophys Acta 1846: 326-41, 2014).

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is associated with one or more mutations in a fms-related tyrosine kinase 3 (FLT3), nucleophosmin (NPM1), isocitrate dehydrogenase 1(IDH1), isocitrate dehydrogenase 2 (IDH2), DNA (cytosine-5)-methyltransferase 3 (DNMT3A), CCAAT/enhancer binding protein alpha (CEBPA), U2 small nuclear RNA auxiliary factor 1(U2AF1), enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), structural maintenance of chromosomes 1A (SMC1A) and structural maintenance of chromosomes 3 (SMC3).

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is associated with one or more mutations in fms-related tyrosine kinase 3 (FLT3).

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is associated with FLT3-ITD.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is associated with one or more mutations in isocitrate dehydrogenase 1(IDH1) or isocitrate dehydrogenase 2 (IDH2).

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is associated with mutations R132H, R132X or R100Q/R104V/F108L/R119Q/I130V in isocitrate dehydrogenase 1 (IDH1).

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is associated with mutations R140Q and R172 in isocitrate dehydrogenase 2 (IDH2).

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is AML with multilineage dysplasia.

AML associated with multilineage dysplasia is characterized by dysplasia in two or more myeloid cell lineage, and by at least 20% increased blasts in either the blood or bone marrow.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is therapy-related AML.

Therapy-related AML is a result of prior chemotherapy and/or radiation therapy, and may occur several years after exposure to the mutagenic agent. More than 90% of

patients with therapy-related AML exhibit chromosomal abnormalities, including those of chromosomes 5 and/or 7.

Chromosomal rearrangements may be identified using well-known methods, for example fluorescent in situ hybridization, karyotyping, Southern blot, or sequencing.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is undifferentiated AML (M0), AML with minimal maturation (M1), AML with maturation (M2), acute myelomonocytic leukemia (M4), acute monocytic leukemia (M5), acute erythroid leukemia (M6), acute megakaryoblastic leukemia (M7), acute basophilic leukemia, acute panmyelosis with fibrosis or myeloid sarcoma.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is in remission.

AML in remission is typically defined as normocellular marrow with less than 5% blasts, normal peripheral blood count with >100,000/mm^3 platelets and >1,000/mm^3 neutrophils.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is relapsed or refractory.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the patient having AML has been treated with idarubicin, cytrabine or hydroxyurea.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is adult AML.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is pediatric AML.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody is administered as a remission induction, post-remission or maintenance therapy.

Various qualitative and/or quantitative methods may be used to determine if a subject has relapsed, is resistant, has developed or is susceptible to developing a resistance to treatment with a drug or a therapeutic. Symptoms that may be associated with relapse and/or resistance include, for example, a decline or plateau of the well-being of the patient, an increase in the size of a tumor or tumor burden, increase in the number of cancer cells, arrested or slowed decline in growth of a tumor or tumor cells, and/or the spread of cancerous cells in the body from one location to other organs, tissues or cells. Re-establishment or worsening of various symptoms associated with tumor may also be an

indication that a subject has relapsed or has developed or is susceptible to developing resistance to a drug or a therapeutic. The symptoms associated with cancer may vary according to the type of cancer. For example, symptoms associated with AML may include weakness, tiredness, feeling dizzy or cold, headaches, frequent nosebleeds, excess bruising or bleeding gums.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody is administered in combination with at least one additional therapeutic.

AML may be treated with cytarabine (cytosine arabinoside, or ara-C) and/or antharcycline drugs such as doxorubicin, daunorubicin, daunomycin, idarubicin and mitoxantrone. Other chemotherapeutic drugs that may be used to treat AML include Hydroxyurea (Hydrea®), Decitabine (Dacogen®), Cladribine (Leustatin®, 2-CdA), Fludarabine (Fludara®), Topotecan, Etoposide (VP-16), 6-thioguanine (6-TG), Corticosteroid drugs, such as prednisone or dexamethasone (Decadron®), methotrexate (MTX), 6-mercaptopurine (6-MP) or Azacitidine (Vidaza®).

Other drugs that may be used to treat AML are all-trans-retinoic acid (ATRA), tretinoin, or Vesanoid® and arsenic trioxide (ATO, Trisenox®). ATRA and arsenic trioxide may be used to treat acute promyelocytic leukemia.

In some embodiments, the anti-CD38 antibody is administered to a patient in combination with cytarabine, daunorubicin/daunomycin, idarubicin, mitoxantrone, hydroxyurea, decitabine, cladribine, fludarabine, topotecan, etoposide 6-thioguanine, corticosteroid, prednisone, dexamethasone, methotrexate, 6-mercaptopurine or azacitidine.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody is administered to a patient in combination with decitabine.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody is administered to a patient in combination with cytarabine and doxorubicin.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the subject has received or will receive radiotherapy.

Radiotherapy may be external beam radiation, intensity modulated radiation therapy (IMRT), focused radiation, or any form of radiosurgery including Gamma Knife, Cyberknife, Linac, and interstitial radiation (e.g. implanted radioactive seeds, GliaSite balloon), and/or with surgery.

Focused radiation methods that may be used include stereotactic radiosurgery, fractionated stereotactic radiosurgery, and intensity-modulated radiation therapy (IMRT). It is apparent that stereotactic radiosurgery involves the precise delivery of radiation to a tumorous tissue, for example, a brain tumor, while avoiding the surrounding non-tumorous, normal tissue. The dosage of radiation applied using stereotactic radiosurgery may vary, typically from 1 Gy to about 30 Gy, and may encompass intermediate ranges including, for example, from 1 to 5, 10, 15, 20, 25, up to 30 Gy in dose. Because of noninvasive fixation devices, stereotactic radiation need not be delivered in a single treatment. The treatment plan may be reliably duplicated day-to-day, thereby allowing multiple fractionated doses of radiation to be delivered. When used to treat a tumor over time, the radiosurgery is referred to as "fractionated stereotactic radiosurgery" or FSR. In contrast, stereotactic radiosurgery refers to a one-session treatment. Fractionated stereotactic radiosurgery may result in a high therapeutic ratio, i.e., a high rate of killing of tumor cells and a low effect on normal tissue. The tumor and the normal tissue respond differently to high single doses of radiation vs. multiple smaller doses of radiation. Single large doses of radiation may kill more normal tissue than several smaller doses of radiation may. Accordingly, multiple smaller doses of radiation can kill more tumor cells while sparing normal tissue. The dosage of radiation applied using fractionated stereotactic radiation may vary from range from 1 Gy to about 50 Gy, and may encompass intermediate ranges including, for example, from 1 to 5, 10, 15, 20, 25, 30, 40, up to 50 Gy in hypofractionated doses. Intensity-modulated radiation therapy (IMRT) may also be used. IMRT is an advanced mode of high-precision three-dimensional conformal radiation therapy (3DCRT), which uses computer-controlled linear accelerators to deliver precise radiation doses to a malignant tumor or specific areas within the tumor. In 3DCRT, the profile of each radiation beam is shaped to fit the profile of the target from a beam's eye view (BEV) using a multileaf collimator (MLC), thereby producing a number of beams. IMRT allows the radiation dose to conform more precisely to the three-dimensional (3-D) shape of the tumor by modulating the intensity of the radiation beam in multiple small volumes. Accordingly, IMRT allows higher radiation doses to be focused to regions within the tumor while minimizing the dose to surrounding normal critical structures. IMRT improves the ability to conform the treatment volume to concave tumor shapes, for example, when the tumor is wrapped around a vulnerable structure, such as the spinal cord or a major organ or blood vessel.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the subject is undergoing hematopoietic stem cell transplantation (HSCT).

In some embodiments of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the HSCT is allogeneic, autologous or synegeneic, i.e. the donor is a twin. Autologous HSCT comprises the extraction of HSC from the subject and freezing of the harvested HSC. After myeloablation, the subject's stored HSC are transplanted into the subject. Allogeneic HSCT involves HSC obtained from an allogeneic HSC donor who has an HLA type that matches the subject.

"Hematopoietic stem cell transplantation" is the transplantation of blood stem cells derived from the bone marrow (in this case known as bone marrow transplantation), blood (such as peripheral blood and umbilical cord blood), or amniotic fluid.

"Undergoing hematopoietic stem cell transplantation" means that the patient did already receive, is receiving or will receive HSCT.

In some embodiments of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the patient has completed chemotherapy and/or radiation therapy prior to HSCT.

Patients may be treated with chemotherapy and/or radiation therapy prior to HSCT (so-called pre-transplant preparation) to eradicate some or all of the patient's hematopoietic cells prior to transplant. The patient may also be treated with immunosuppressants in case of allogeneic HSCT. An exemplary pre-transplant preparation therapy is high-dose melphalan (see for example Skinner *et al.,* Ann Intern Med 140:85-93, 2004; Gertz *et al.,* Bone Marrow Transplant 34: 1025-31, 2004; Perfetti *et al.,* Haematologica 91:1635-43, 2006). The radiation therapy that may be employed in pre-transplant treatment may be carried out according to commonly known protocols in this field. Radiation therapy may also be provided simultaneously, sequentially or separately with the anti-CD38 antibody.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the subject having AML is homozygous for phenylalanine at position 158 of CD16 (FcγRIIIa-158F/F genotype) or heterozygous for valine and pheynylalanine at position 158 of CD16 (FcγRIIIa-158F/V genotype). CD16 is also known as the Fc gamma receptor IIIa (FcγRIIIa) or the low affinity immunoglobulin gamma Fc region receptor III-A isoform. Valine/phenylalanine

(V/F) polymorphism at FcγRIIIa protein residue position 158 has been shown to affect FcγRIIIa affinity to human IgG. Receptor with FcγRIIIa-158F/F or FcγRIIIa-158F/V polymorphisms demonstrates reduced Fc engagement and therefore reduced ADCC when compared to the FcγRIIIa-158V/V. The lack of or low amount of fucose on human N-linked oligosaccharides improves the ability of the antibodies to induce ADCC due to improved binding of the antibodies to human FcγRIIIa (CD16) (Shields *et al.*, J Biol Chem 277:26733-40, 2002). Patients can be analyzed for their FcγRIIIa polymorphism using routine methods.

The invention also provides for the method of treating a subject having AML, comprising administering to a patient in need thereof an anti-CD38 antibody that binds to the region SKRNIQFSCKNIYR (SEQ ID NO: 2) and the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1), wherein the subject is homozygous for phenylalanine at position 158 of CD16 or heterozygous for valine and pheynylalanine at position 158 of CD16.

The invention also provides an anti-CD38 antibody for use in treating a subject having AML, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody for use in treating a subject having AML, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3)

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with dacogen, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3)

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with dacogen, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with dacogen, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with dacogen, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with dacogen, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with dacogen, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3)

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3)

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3)

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with dacogen, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with dacogen, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with dacogen, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with dacogen, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with dacogen, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with dacogen, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 15 and the VL of SEQ ID NO: 16 for use in treating a subject having AML, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 15 and the VL of SEQ ID NO: 16 for use in treating a subject having AML, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 15 and the VL of SEQ ID NO: 16 for use in treating a subject having AML, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 15 and the VL of SEQ ID NO: 16 for use in treating a subject having AML, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 15 and the VL of SEQ ID NO: 16 for use in treating a subject having AML, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 15 and the VL of SEQ ID NO: 16 for use in treating a subject having AML, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 17 and the VL of SEQ ID NO: 18 for use in treating a subject having AML, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 17 and the VL of SEQ ID NO: 18 for use in treating a subject having AML, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 17 and the VL of SEQ ID NO: 18 for use in treating a subject having AML, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 17 and the VL of SEQ ID NO: 18 for use in treating a subject having AML, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 17 and the VL of SEQ ID NO: 18 for use in treating a subject having AML, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 17 and the VL of SEQ ID NO: 18 for use in treating a subject having AML, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 19 and the VL of SEQ ID NO: 20 for use in treating a subject having AML, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 19 and the VL of SEQ ID NO: 20 for use in treating a subject having AML, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 19 and the VL of SEQ ID NO: 20 for use in treating a subject having AML, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 19 and the VL of SEQ ID NO: 20 for use in treating a subject having AML, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 19 and the VL of SEQ ID NO: 20 for use in treating a subject having AML, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 19 and the VL of SEQ ID NO: 20 for use in treating a subject having AML, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 21 and the VL of SEQ ID NO: 22 for use in treating a subject having AML, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 21 and the VL of SEQ ID NO: 22 for use in treating a subject having AML, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 21 and the VL of SEQ ID NO: 22 for use in treating a subject having AML, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 21 and the VL of SEQ ID NO: 22 for use in treating a subject having AML, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 21 and the VL of SEQ ID NO: 22 for use in treating a subject having AML, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 21 and the VL of SEQ ID NO: 22 for use in treating a subject having AML, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

Administration/ Pharmaceutical Compositions

In the methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibodies may be provided in suitable pharmaceutical compositions comprising the anti-CD38 antibody and a pharmaceutically acceptable carrier. The carrier may be diluent, adjuvant, excipient, or vehicle with which the anti-CD38 antibody is administered. Such vehicles may be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. For example, 0.4% saline and 0.3% glycine can be used. These solutions are sterile and generally free of particulate matter. They may be sterilized by conventional, well-known sterilization techniques (*e.g.*, filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such

as pH adjusting and buffering agents, stabilizing, thickening, lubricating and coloring agents, etc. The concentration of the molecules or antibodies of the invention in such pharmaceutical formulation may vary widely, *i.e.*, from less than about 0.5%, usually to at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on required dose, fluid volumes, viscosities, etc., according to the particular mode of administration selected. Suitable vehicles and formulations, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in e.g. Remington: The Science and Practice of Pharmacy, 21st Edition, Troy, D.B. ed., Lipincott Williams and Wilkins, Philadelphia, PA 2006, Part 5, Pharmaceutical Manufacturing pp 691-1092, see especially pp. 958-989.

The mode of administration of the anti-CD38 antibody in the methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, may be any suitable route such as parenteral administration, e.g., intradermal, intramuscular, intraperitoneal, intravenous or subcutaneous, pulmonary, transmucosal (oral, intranasal, intravaginal, rectal) or other means appreciated by the skilled artisan, as well known in the art.

The anti-CD38 antibody in the methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, may be administered to a patient by any suitable route, for example parentally by intravenous *(i.v.)* infusion or bolus injection, intramuscularly or subcutaneously or intraperitoneally. *i.v.* infusion may be given over for example 15, 30, 60, 90, 120, 180, or 240 minutes, or from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 hours.

The dose given to a patient having AML is sufficient to alleviate or at least partially arrest the disease being treated ("therapeutically effective amount") and may be sometimes 0.005 mg to about 100 mg/kg, e.g. about 0.05 mg to about 30 mg/kg or about 5 mg to about 25 mg/kg, or about 4 mg/kg, about 8 mg/kg, about 16 mg/kg or about 24 mg/kg , or for example about 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mg/kg, but may even higher, for example about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, 50, 60, 70, 80, 90 or 100 mg/kg.

A fixed unit dose may also be given, for example, 50, 100, 200, 500 or 1000 mg, or the dose may be based on the patient's surface area, e.g., 500, 400, 300, 250, 200, or 100 mg/m^2. Usually between 1 and 8 doses, (e.g., 1, 2, 3, 4, 5, 6, 7 or 8) may be administered to treat AML, but 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more doses may be given.

The administration of the anti-CD38 antibody in the methods of the invention described herein, and in some embodiments of each and every one of the numbered

embodiments listed below, may be repeated after one day, two days, three days, four days, five days, six days, one week, two weeks, three weeks, one month, five weeks, six weeks, seven weeks, two months, three months, four months, five months, six months or longer. Repeated courses of treatment are also possible, as is chronic administration. The repeated administration may be at the same dose or at a different dose. For example, the anti-CD38 antibody in the methods of the invention may be administered at 8 mg/kg or at 16 mg/kg at weekly interval for 8 weeks, followed by administration at 8 mg/kg or at 16 mg/kg every two weeks for an additional 16 weeks, followed by administration at 8 mg/kg or at 16 mg/kg every four weeks by intravenous infusion.

The anti-CD38 antibodies may be administered in the methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, by maintenance therapy, such as, e.g., once a week for a period of 6 months or more.

For example, anti-CD38 antibodies in the methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, may be provided as a daily dosage in an amount of about 0.1-100 mg/kg, such as 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively, at least one of week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 after initiation of treatment, or any combination thereof, using single or divided doses of every 24, 12, 8, 6, 4, or 2 hours, or any combination thereof.

Anti-CD38 antibodies in the methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, may also be administered prophylactically in order to reduce the risk of developing cancer, delay the onset of the occurrence of an event in cancer progression, and/or reduce the risk of recurrence when a cancer is in remission. This may be especially useful in patients wherein it is difficult to locate a tumor that is known to be present due to other biological factors.

The anti-CD38 antibody in the methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, may be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional protein preparations and well known lyophilization and reconstitution techniques can be employed.

The anti-CD38 antibody in the methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, may be administered in combination with all-trans retinoic acid (ATRA).

ATRA may be provided as a dosage of 45 mg/m^2/day PO or 25 mg/m^2/day PO.

The anti-CD38 antibody in the methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, may be administered in combination with dacogen.

Dacogen may be administered for a minimum of 4 cycles repeated every 6 weeks at 15 mg/m^2 *i.v.* over 3 hours repeated every 8 hours for 3 days. Alternatively, dacogen may be administered 20 mg/m^2 *i.v.* over 1 hour repeated daily for 5 days, and the cycle repeated every 4 weeks.

The anti-CD38 antibody in the methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, may be administered in combination with cytrabine and doxorubicin.

Cytarabine may be administered 2 to 3 g/m^2 *i.v.* over 1-3 hours every twelve hours for up to 12 doses.

Doxorubicin may be administered 40 to 60 mg/m^2 *i.v.* every 21 to 28 days, or 60 to 75 mg/m^2 *i.v.* once every 21 days.

Anti-CD38 antibody may be administered together with any form of radiation therapy including external beam radiation, intensity modulated radiation therapy (IMRT) and any form of radiosurgery including Gamma Knife, Cyberknife, Linac, and interstitial radiation (e.g. implanted radioactive seeds, GliaSite balloon), and/or with surgery.

While having described the invention in general terms, the embodiments of the invention will be further disclosed in the following examples that should not be construed as limiting the scope of the claims.

Further embodiments of the invention

Set out below are certain further embodiments of the invention according to the disclosures elsewhere herein. Features from embodiments of the invention set out above described as relating to the invention disclosed herein also relate to each and every one of these further numbered embodiments.

1. An anti-CD38 antibody for use in treating a subject having acute myeloid leukemia (AML).

2. An anti-CD38 antibody for use in treating a subject having AML, in combination with a second therapeutic agent, wherein the second therapeutic agent
    a. is optionally cytarabine, daunorubicin, idarubicin, mitoxantrone, hydroxyurea, decitabine, cladribine, fludarabine, topotecan, etoposide 6-thioguanine, corticosteroid, prednisone, dexamethasone, methotrexate, 6-mercaptopurine, azacitidine, arsenic trioxide or all-trans retinoic acid; and/or
    b. increases surface expression of CD38.
3. A combination of an anti-CD38 antibody and all-trans retinoic acid for use in treating a subject having AML.
4. A combination of an anti-CD38 antibody and decitabine for use in treating a subject having AML.
5. A combination of an anti-CD38 antibody and cytarabine and/or doxorubicin for use in treating a subject having AML.
6. The anti-CD38 antibody for use according to embodiment 1 or 2, or the combination according to embodiment 3-5, wherein the anti-CD38 antibody competes for binding to CD38 with an antibody comprising a heavy chain variable region (VH) of SEQ ID NO: 4 and a light chain variable region (VL) of SEQ ID NO: 5.
7. The anti-CD38 antibody for use according to embodiment 1, 2 or 6, or the combination according to embodiment 3-6, wherein the anti-CD38 antibody induces killing of AML cells that express CD38 by apoptosis.
8. The anti-CD38 antibody for use according to embodiment 1, 2, 6 or 7 or the combination according to embodiment 3-7, wherein the anti-CD38 antibody binds to the region SKRNIQFSCKNIYR (SEQ ID NO: 2) and the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1).
9. The anti-CD38 antibody for use according to embodiment 1, 2, 6-8, or the combination according to embodiment 3-8, wherein the anti-CD38 antibody:
    a. is of IgG1, IgG2, IgG3 or IgG4 isotype;
    b. has a biantennary glycan structure with fucose content of about 50%, 40%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 14%, 13%, 12%, 11% 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1% or 0%; or
    c. comprise a substitution in the antibody Fc at amino acid positions 256, 290, 298, 312, 356, 330, 333, 334, 360, 378 or 430, when residue numbering according to the EU index.

10. The anti-CD38 antibody for use according to embodiment 1, 2, 6-9, or the combination according to embodiment 3-9, wherein the anti-CD38 antibody comprises

a. the heavy chain complementarity determining regions (HCDR) 1 (HCDR1), 2 (HCDR2) and 3 (HCDR3) sequences of SEQ ID NOs: 6, 7 and 8, respectively;

b. the light chain complementarity determining regions (LCDR) 1 (LCDR1), 2 (LCDR2) and 3 (LCDR3) sequences of SEQ ID NOs: 9, 10 and 11, respectively;

c. HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 6, 7, 8, 9, 10 and 11, respectively;

d. the heavy chain variable region (VH) of SEQ ID NO: 4 and the light chain variable region (VL) of SEQ ID NO: 5;

e. a heavy chain comprising an amino acid sequence that is 95%, 96%, 97%, 98% or 99% identical to that of SEQ ID NO: 12 and a light chain comprising an amino acid sequence that is 95%, 96%, 97%, 98% or 99% identical to that of SEQ ID NO: 13; or

f. the heavy chain of SEQ ID NO: 12 and the light chain of SEQ ID NO: 13.

11. The anti-CD38 antibody for use according to embodiment 1, 2, 6-10, or the combination according to embodiment 3-10, wherein AML with at least one genetic abnormality, AML with multilineage dysplasia, therapy-related AML, undifferentiated AML, AML with minimal maturation, AML with maturation, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia, acute basophilic leukemia, acute panmyelosis with fibrosis or myeloid sarcoma.

12. The anti-CD38 antibody for use according to embodiment 1, 2, 6-11, or the combination according to embodiment 3-11, wherein the anti-CD38 antibody is administered as a remission induction, post-remission or maintenance therapy.

13. The anti-CD38 antibody for use according to embodiment 1, 2, 6-12, or the combination according to embodiment 3-12, wherein the at least one genetic abnormality is a translocation between chromosomes 8 and 21, a translocation or an inversion in chromosome 16, a translocation between chromosomes 15 and 17, changes in chromosome 11, or mutation in fms-related tyrosine kinase 3 (FLT3), nucleophosmin (NPM1), isocitrate dehydrogenase 1(IDH1), isocitrate dehydrogenase 2 (IDH2), DNA (cytosine-5)-methyltransferase 3 (DNMT3A),

CCAAT/enhancer binding protein alpha (CEBPA), U2 small nuclear RNA auxiliary factor 1(U2AF1), enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), structural maintenance of chromosomes 1A (SMC1A) or structural maintenance of chromosomes 3 (SMC3).

14. The anti-CD38 antibody for use according to embodiment 1, 2, 6-13, or the combination according to embodiment 3-13, wherein the at least one genetic abnormality is a translocation t(8; 21)(q22; q22), an inversion inv(16)(p13; q22), a translocation t(16; 16)(p13; q22), a translocation t(15; 17)(q22; q12), a mutation FLT3-ITD, mutations R132H or R100Q/R104V/F108L/R119Q/I130V in IDH1 or mutations R140Q or R172 in IDH2.

15. The anti-CD38 antibody for use according to embodiment 1, 2, 6-14, or the combination according to embodiment 3-14, wherein the anti-CD38 antibody and the at least one therapeutic agent are administered simultaneously, sequentially or separately.

16. The anti-CD38 antibody for use according to embodiment 1, 2, 6-15, or the combination according to embodiment 3-15, wherein

a. the subject is further treated or has been treated with radiotherapy; or
b. the subject has received hematopoietic stem cell transplantation.

Examples

Example 1. Efficacy of daratumumab in AML cell lines

Several AML cell lines were used to evaluate surface expression of CD38 and possible efficacy of daratumumab in inducing AML cell killing. Expression of complement inhibitory proteins (CIP) CD46, CD55 and CD59 in the AML cell lines was assessed to evaluate possible correlation between expression of CIP and CDC.

Methods:

ADCC

In vitro ADCC assays were performed using AML tumor cell lines and Peripheral Blood Mononuclear Cells (PBMC) as effector cells at a ratio of 50:1. One hundred µl of target (tumor) cells ($1x10^4$ cells) were added to wells of 96-well U-bottom plates. An additional 100 µl was added with or without antibody, and the plates were incubated for 30 minutes at room temperature (RT) before adding effector cells (PBMC). Seventy five µl of PBMCs at concentration $6.66x10^6$ cells/ml was added to the wells of the plates, and the

plates were incubated at 37°C for 6 hours. Plates were centrifuged at 250 g for 4 minutes, 50 µl of supernatant removed per well and cell lysis was measured using the CellTiter-Glo® assay (Promega).

CDC

Target cells were harvested and adjusted to a concentration of $80x10^4$cells/ml. Twelve µl of target cells were added to wells of a 96-well plate, and serial dilution of antibodies added onto the cells. The wells were incubated for 15 minutes, after which human serum high in complement was added at a final concentration of 10%. Reaction mixture was incubated for 21/2 hours at 37°C, and cell lysis was measured using the CellTiter-Glo® assay (Promega).

Apoptosis

One ml of target cells (5×10^5 cells/ml) were added to the well of a 24-well plate, together with test antibody (1 µg/ml) in the presence or absence of rabbit anti-huIgG (10 µg/ml; F(ab')2 Fcγ-specific). Cells were incubated for 22 hours (5% CO2, 37°C). Thereafter, cells were harvested (1000 rpm, 5 min) and washed twice in PBS (1000 rpm, 5 min). Cells were resuspended in 250 µl binding buffer (Annexin-V Apoptosis kit, BD Biosciences) according to manufacturer's instruction, followed by flow cytometry analysis.

Apoptosis was measured by both early and late apoptosis (Q2 and Q3 in Figure 1A and Figure 1B).

CD38, CD46, CD55 and CD59 surface expression

Expression of receptors was analyzed by flow cytometry. The CD38 receptor number per cell was estimated using MESF kit using PE-labeled anti-CD38 antibody (R&D Systems). The receptor numbers were calculated as follows: Specific MESF/ABC = MESF/ABC (Test Antibody) — MESF/ABC (Isotype control antibody).

CD46, CD55 and CD59, surface expression was detected using FITC anti-human CD46, PE-anti-human CD55 and PE-anti-human CD59 antibodies (Beckton Dickinson) expressed as median fluorescent intensity (MFI).

Results

Table 1 shows the results of the experiments. **Figure 1** shows representative flow cytometry results of daratumumab-induced apoptosis in NB-4 cell line without **(Figure 1A)** or with **(Figure 1B)** crosslinking antibody. In this cell line, daratumumab induced apoptosis to a similar degree independent of the presence of the crosslinking agent (19.2% vs 18.3%).

In the AML cell lines, daratumumab did not induce significant ADCC or CDC; instead; daratumumab induced AML cell killing by apoptosis. In addition, no direct correlation was observed between CD38 expression and the extent of ADCC and CDC. The levels of complement inhibitory proteins (CIP) (CD46, CD55 and CD59) were evaluated to determine if these proteins affected CDC in response to daratumumab but no direct correlation was observed between CDC and CIP expression.

Table 1.

Cell line	CD38 #/cell	CD46 MFI	CD55 MFI	CD59 MFI	Apoptosis	CDC	ADCC
HL-60	64,50	ND	ND	ND	ND	ND	ND
Kasumi-1	120.2	ND	ND	ND	ND	ND	ND
ML-2	1,253.27	21.53	195.2	0.98	5%	0%	6.30%
MOLM-13	5,634.29	35.53	173.2	9.45	10-15%	0%	9.40%
MOLM-16	52,461.11	42.18	886.4	350.42	20-30%	5%	18.20%
MV-4-11	5,700.05	207.17	395.42	43.94	10-12%	0%	2.30%
NB4	9,370.73	58.25	345.4	66.2	18%	4%	18.30%
THP-1	39,488.19	58.7	375	27.1	5-7%	5%	11.30%

ND: not done
MFI: mean fluorescence intensity

Example 2. ATRA induces CD38 expression on AML cells

Effect of ATRA on CD38 surface expression was assessed in NB-4 AML cell line. Tumor cells were incubated at 37°C for 24 hours in the presence or absence of 10 nM or 100 nM ATRA. After 24 hour incubation, the cells were harvested and stained for CD38. ATRA induced ~10-fold increase in CD38 receptors in the NB-4 cell line. CD38 surface expression was assessed using FACS using PE-labeled anti-CD38 antibody (R&D Systems) (Table 2).

Table 2.

Treatment	PE-CD38 molecules/cell
DMSO	17238
10 nM ATRA	185737
100 nM ATRA	210570

Example 3. Efficacy of daratumumab in patient-derived xenograft (PDX) models

Methods

Patient tumor models AML 3406, AML 7577 and AML 8096 were used in the study.

AML3406 model: Patient tumor cells were positive for FLT-3ITD. Patient has a history of polycythemia versa, and received idarubicin/ cytrabine for induction chemotherapy. Patient also received Hudrea® (hydroxyurea).

AML 7577 model: Leukemic cells were collected from a 69-year old male with AML (FAB subtype M5). Patient had normal karyotype and following mutations: IDH2(R140Q); FLT3-ITD; DNMT3A R882H, NPM1, CEBPA insertion (SNP). Patient has a history of polycythemia versa, and received idarubicin/ cytrabine for induction chemotherapy. Patient also received Hudrea® (hydroxyurea).

AML 8096 model: Leukemic cells were collected from a 21-year old male with AML (FAB subtype M2). White blood cell count was 20X10e^9/L, from which 70% were blast cells. Patient had normal karyotype with wild type TP53, FLT3, NPM1, and insertion 570-587, 3GCACCC>4GCACCC in CEBPA exon1. Patient has a history of polycythemia versa, and received idarubicin/ cytrabine for induction chemotherapy. Patient also received Hudrea® (hydroxyurea).

5 million AML MNCs were T-cell depleted and transplanted via tail vein into 6-8 weeks old sub-lethaly irradiated NSG mice (n=10 per group). 4 to 6 weeks post-engraftment, bone marrow aspirates were collected from each mouse and were analyzed by flow cytometry to determine the level of leukemia engraftment (% of human $CD45^+$ $CD33^{+/-}$ cells). Based on engraftment levels, mice were randomized and conditioned with either IgG1 or daratumumab (DARA, pre-dosing at 0.5 mg/kg). 24 hours later, mice were untreated (Ctrl) or treated for 5 consecutive weeks with DARA or IgG1 alone (i.p, 10mg/kg once a week). 2-3 days after the last treatment, mice were sacrificed and bone marrow, spleen, peripheral blood and plasma were collected for analysis. Flow cytometry was performed to assess percentage of human $CD45^+CD33^+$ cells in the BM, SPL and PB of 3 AML patients engrafted in NSG mice (AML 3406 model: **Figure 2A;** AML 7577 model: **Figure 2B,** AML 8096 model: **Figure 2C**) and absolute number of the human $CD45^+CD33^+$ cells in bone marrow **(Figure 3A),** spleen **(Figure 3B)** and peripheral blood **(Figure 3C)** of one representative AML patient.

Results

Figure 2A, Figure 2B and Figure 2C show the efficacy of daratumumab in the AML 3406 model, AML 7577 model and the AML 8096 model, respectively, assessed by reduction in % leukemic $CD45^+CD33^+$ cells in bone marrow, spleen or peripheral blood. Daratumumab reduced tumor burden in spleen and peripheral blood in the AML 3406 model (Figure 2A), in peripheral blood in the AML 7577 model (Figure 2B), and in spleen in the AML 8096 model (Figure 2C).

Efficacy of daratumumab was also assessed by measuring daratumumab-induced reduction in total leukemic burden in bone marrow (Figure 3A), spleen (Figure 3B) and blood (Figure 3C) in the AML 3406 model. Daratumumab significantly reduced total leukemic burden in the AML 3406 model in spleen (Figure 3B) and in peripheral blood (Figure 3C).

Example 4. Effect of daratumumab on CD38 expression on AML blasts

Effect of daratumumab on CD38 expression on leukemic blasts was assessed in one representative AML model described in Example 3 after 5 weeks of treatment with daratumumab or isotype control using PE-labeled anti-CD38 antibody (R&D Systems).

Results

Figure 4A shows that treatment with daratumumab reduced expression of CD38 on leukemia blasts ($CD45^+CD33^+$ positive cells) in bone marrow, spleen and peripheral blood. **Figure 4B** shows that percentage of CD38-positive AML blasts were reduced after 5 weeks of treatment.

Example 5. Efficacy of daratumumab combination therapy in patient-derived xenograft (PDX) models

Efficacy of daratumumab in combination with dacogen or cytarabine and doxorubicin was assessed after 5 weeks of treatment.

5 million AML MNCs were T-cell depleted and transplanted via tail vein into 6-8 weeks old NSG mice (n=10 per group). 4 to 6 weeks post-engraftment, bone marrow aspirates were collected from each mouse and were analyzed by flow cytometry to determine the level of leukemia engraftment (% of human $CD45^+$ $CD33^{+/-}$ cells). Based on engraftment levels, mice were equally randomized and conditioned with either IgG1 or DARA (pre-dosing at 0.5 mg/kg). 24 hours later, mice were treated with IgG1 alone (i.p, 10mg/kg) once a week for five weeks, with DARA alone (i.p,10 mg/kg) once a week for five weeks, with decitabine alone (DAC) (0.5 mg/kg/day, i.p. for 3 consecutive days) for five weeks, with DAC + DARA (each week will consist of 3 consecutive days of DAC followed by DARA 2 days later), with a combination of cytarabine (i.v, 50 mg/kg) and doxorubicin (i.v, 1.5 mg/kg) (3 consecutive days doxorubicin (i.v, 1.5 mg/kg) plus cytarabine (50 mg/kg) for 3 days) with or without DARA . 2-3 days after the last treatment, mice were sacrificed and bone marrow, spleen, peripheral blood and plasma were collected for analysis. Flow cytometry was performed to assess percentage of human $CD45^+CD33^+$ cells in the bone marrow **(Figure 5A)**, spleen **(Figure 5B)** and peripheral blood **(Figure 5C)** of one AML patient engrafted in NSG mice.

CD38 expression (expressed as mean fluorescence intensity, MFI) was evaluated in the bone marrow **(Figure 6A)**, spleen **(Figure 6B)** and peripheral blood **(Figure 6C)** after 5 week treatment with the indicated drugs.

We claim:

1) A method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody for a time sufficient to treat AML.
2) The method of claim 1, wherein the anti-CD38 antibody competes for binding to human CD38 of SEQ ID NO: 1 with an antibody comprising a heavy chain variable region (VH) of SEQ ID NO: 4 and a light chain variable region (VL) of SEQ ID NO: 5.
3) The method of claim 1 or 2, wherein the anti-CD38 antibody binds to the region SKRNIQFSCKNIYR (SEQ ID NO: 2) and the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1).
4) The method of any of the claims 1-3, wherein the anti-CD38 antibody induces killing of AML cells that express CD38 by apoptosis.
5) The method of any of the claims 1-4, wherein the anti-CD38 antibody is of IgG1, IgG2, IgG3 or IgG4 isotype.
6) The method of any of the claism 1-5, wherein the anti-CD38 antibody comprises the heavy chain complementarity determining regions (HCDR) 1 (HCDR1), 2 (HCDR2) and 3 (HCDR3) sequences of SEQ ID NOs: 6, 7 and 8, respectively, and the light chain complementarity determining regions (LCDR) 1 (LCDR1), 2 (LCDR2) and 3 (LCDR3) sequences of SEQ ID NOs: 9, 10 and 11, respectively.
7) The method of claim 6, wherein the anti-CD38 antibody comprises the heavy chain variable region (VH) of SEQ ID NO: 4 and the light chain variable region (VL) of SEQ ID NO: 5.
8) The method of claim 7, wherein the anti-CD38 antibody comprises the heavy chain of SEQ ID NO: 12 and the light chain of SEQ ID NO: 13.
9) The method of any of the claims 1-5, wherein the anti-CD38 antibody comprises the VH and the VL of SEQ ID NOs: 15 and 16, respectively.
10) The method of any of the claims 1-5, wherein the anti-CD38 antibody comprises the VH and the VL of SEQ ID NOs: 17 and 18, respectively.
11) The method of any of the claims 1-5, wherein the anti-CD38 antibody comprises the VH and the VL of SEQ ID NOs: 19 and 20, respectively.
12) The method of any of the claims 1-5, wherein the anti-CD38 antibody comprises the VH and the VL of SEQ ID NOs: 21 and 22, respectively.

13) The method of any of the claims 1-12, wherein AML is AML with at least one genetic abnormality, AML with multilineage dysplasia, therapy-related AML, undifferentiated AML, AML with minimal maturation, AML with maturation, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia, acute basophilic leukemia, acute panmyelosis with fibrosis or myeloid sarcoma.

14) The method of claim 13, wherein the at least one genetic abnormality is a translocation between chromosomes 8 and 21, a translocation or an inversion in chromosome 16, a translocation between chromosomes 15 and 17, changes in chromosome 11, or mutation in fms-related tyrosine kinase 3 (FLT3), nucleophosmin (NPM1), isocitrate dehydrogenase 1(IDH1), isocitrate dehydrogenase 2 (IDH2), DNA (cytosine-5)-methyltransferase 3 (DNMT3A), CCAAT/enhancer binding protein alpha (CEBPA), U2 small nuclear RNA auxiliary factor 1(U2AF1), enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), structural maintenance of chromosomes 1A (SMC1A) or structural maintenance of chromosomes 3 (SMC3).

15) The method of claim 13, wherein the at least one genetic abnormality is a translocation t(8; 21)(q22; q22), an inversion inv(16)(p13; q22), a translocation t(16; 16)(p13; q22), a translocation t(15; 17)(q22; q12), a mutation FLT3-ITD, mutations R132H or R100Q/R104V/F108L/R119Q/I130V in IDH1 or mutations R140Q or R172 in IDH2.

16) The method of any of the claims 1-15, wherein AML is refractory or relapsed.

17) The method of any of the claims 1-16, wherein the anti-CD38 antibody is administered as a remission induction, post-remission or maintenance therapy.

18) The method of any of the claims 1-17, wherein the anti-CD38 antibody is administered in combination with at least one second therapeutic agent.

19) The method of claim 18, wherein the at least one second therapeutic agent is cytarabine, daunorubicin, idarubicin, mitoxantrone, hydroxyurea, decitabine, cladribine, fludarabine, topotecan, etoposide 6-thioguanine, corticosteroid, prednisone, dexamethasone, methotrexate, 6-mercaptopurine, azacitidine, arsenic trioxide or all-trans retinoic acid.

20) The method of claim 18, wherein the at least one second therapeutic agent is all-trans retinoic acid, cytarabine, decitabine or doxorubicin.

21) The method of any of the claims 17-20, wherein the anti-CD38 antibody and the at least one second therapeutic agent are administered simultaneously, sequentially or separately.

22) The method of any of the claims 17-21, wherein the at least one second therapeutic agent increases surface expression of CD38 on AML cells.

23) The method of any of the claims 1-22, wherein the subject is further treated or has been treated with radiotherapy.

24) The method of any of the claims 1-23, wherein the subject is undergoing hematopoietic stem cell transplantation (HSCT).

25) The method of claim 24, wherein the HSCT is allogeneic, autologous or syngeneic.

26) The method of claim 25, wherein the HSCT comprises transplantation of blood stem cells derived from bone marrow, blood or amniotic fluid.

Figure 1A.

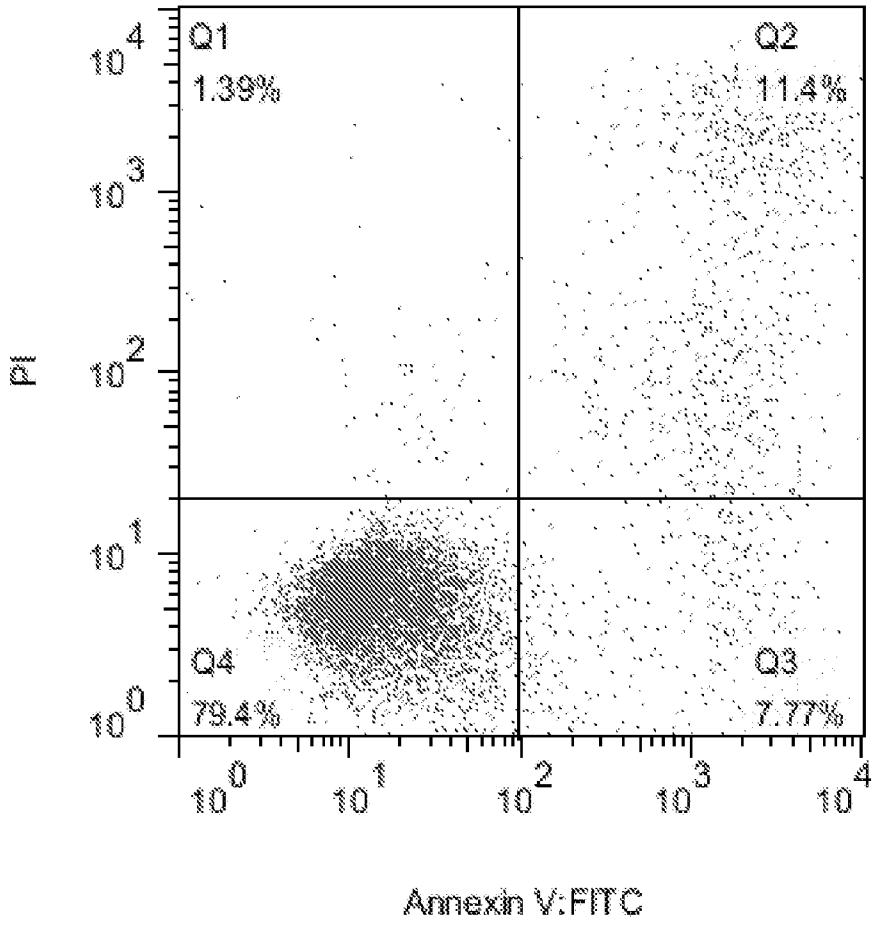


Figure 1B.

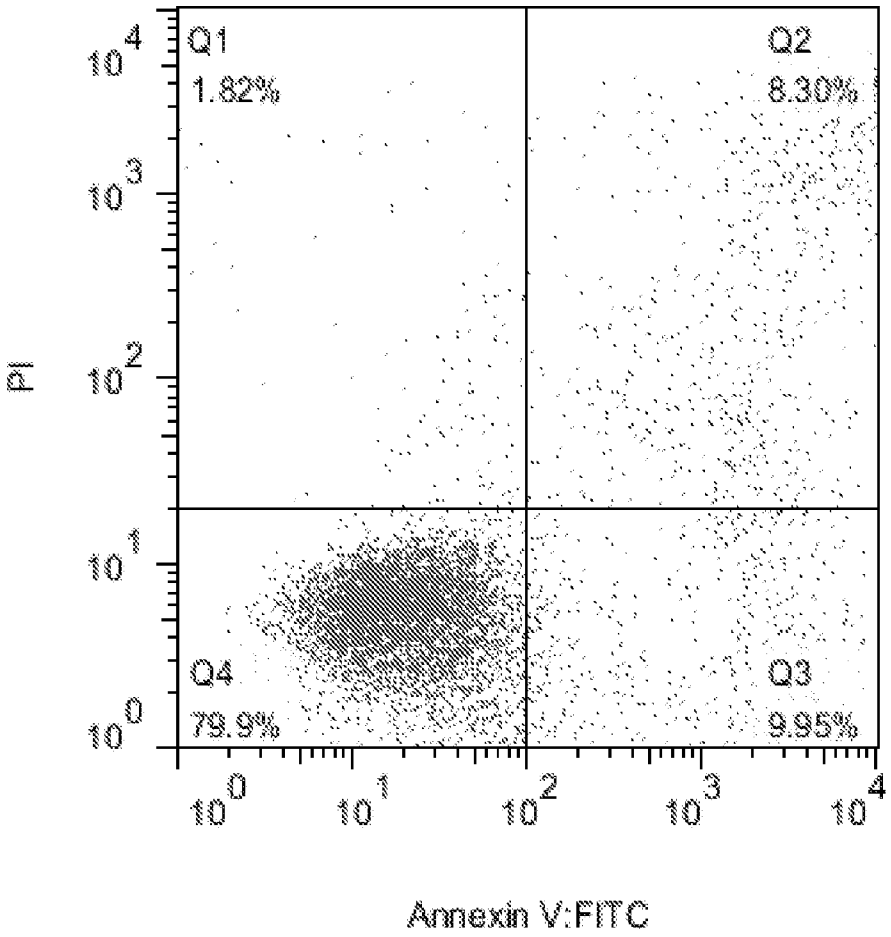


Figure 2A.

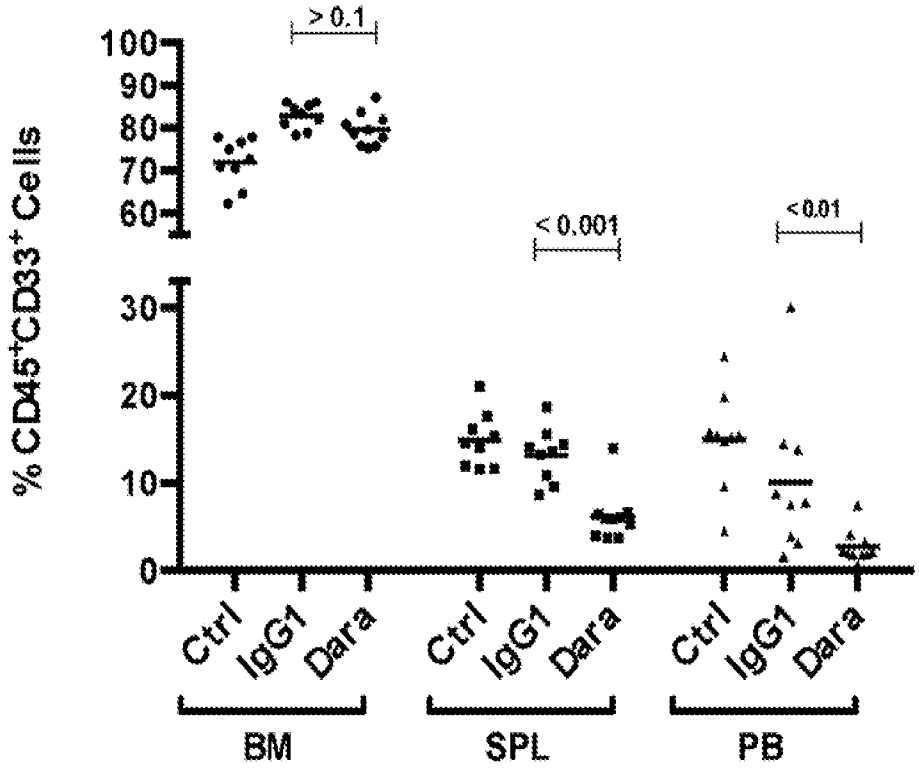

% CD45+CD33+ Cells
100
90
80
70
60
30
20
10
0
> 0.1
< 0.001
< 0.01
Ctrl
IgG1
Dara
Ctrl
IgG1
Dara
Ctrl
IgG1
Dara
BM
SPL
PB

Figure 2B.

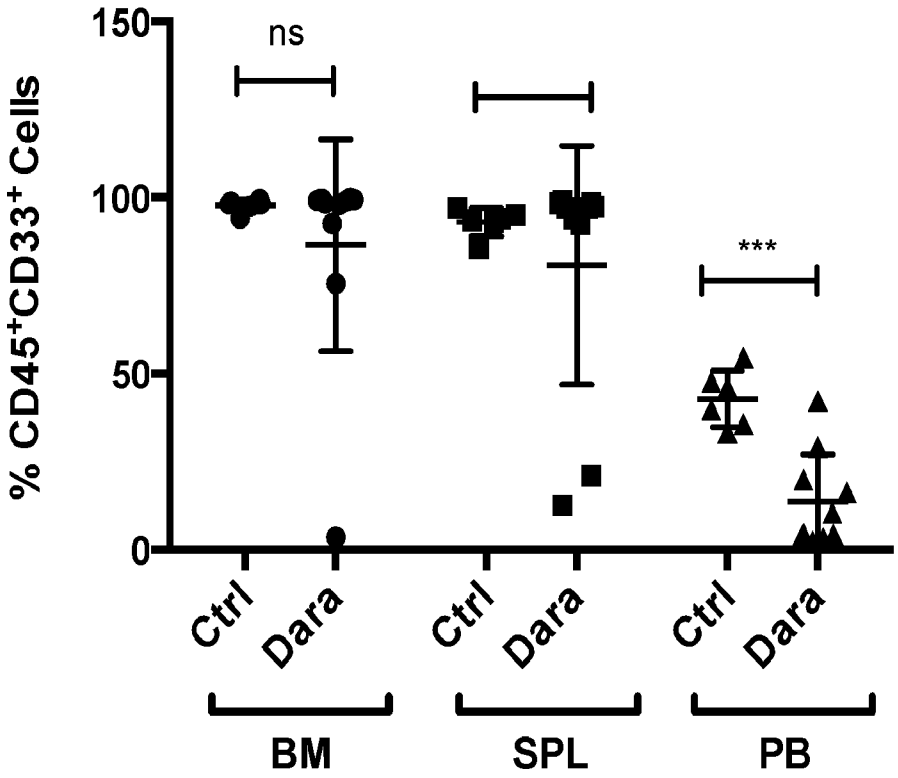

150
100
50
0
% CD45+CD33+ Cells
ns
***
Ctrl
Dara
Ctrl
Dara
Ctrl
Dara
BM
SPL
PB

Figure 2C.

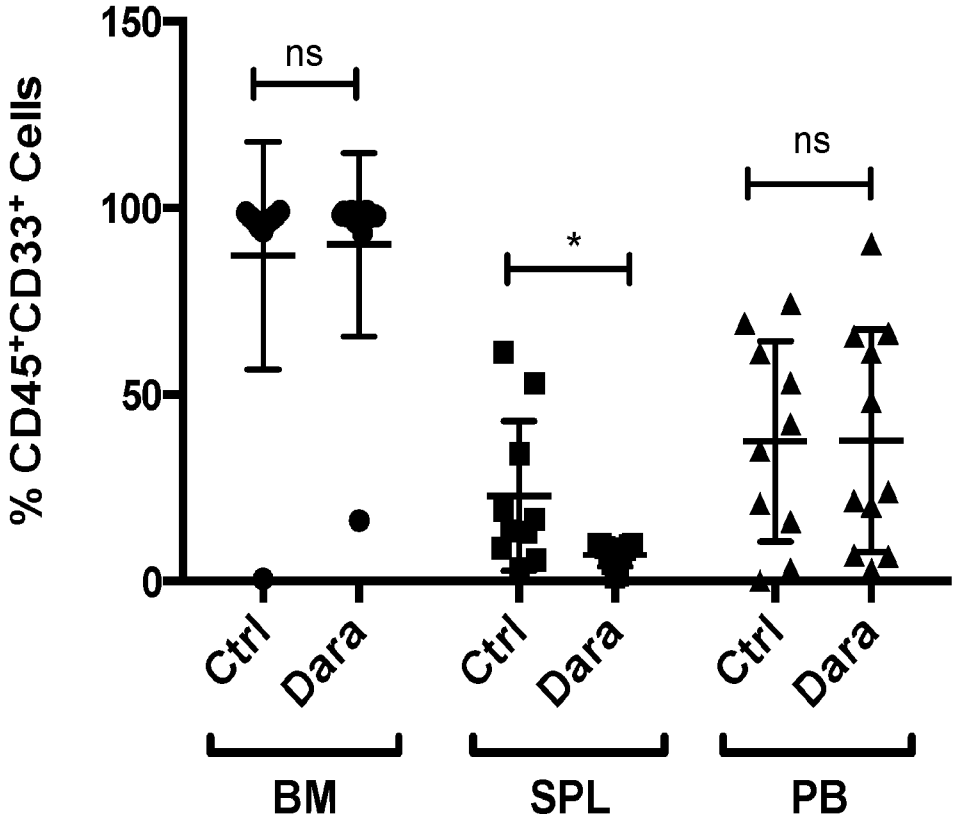

150
100
50
0
% CD45+CD33+ Cells
ns
*
ns
Ctrl
Dara
Ctrl
Dara
Ctrl
Dara
BM
SPL
PB

Figure 3A.

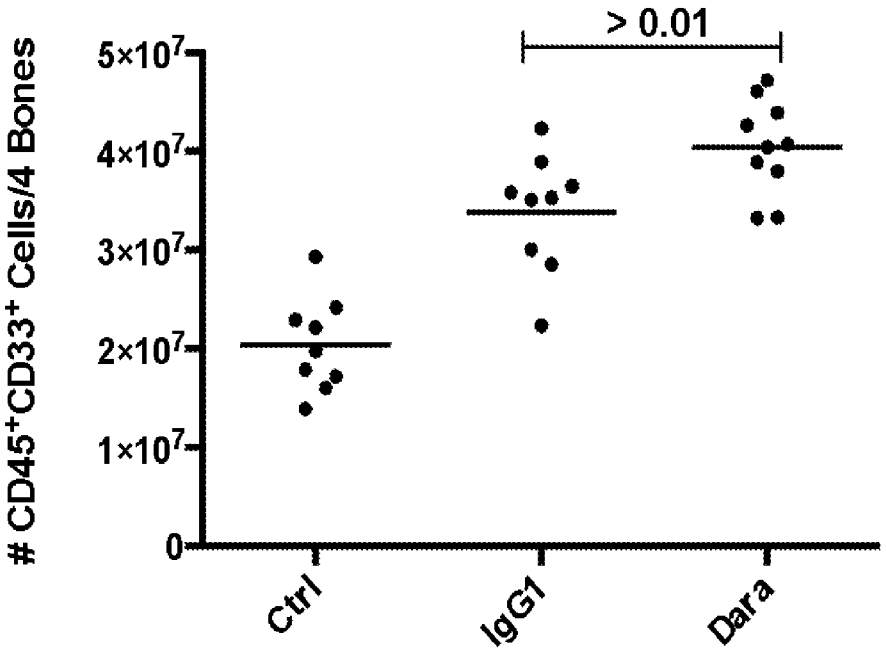
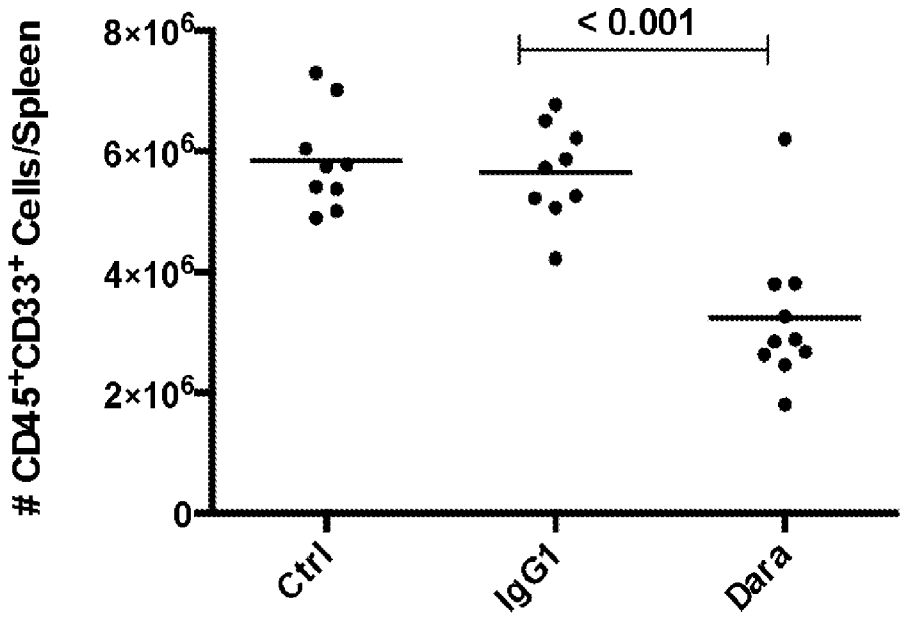

CD45+CD33+ Cells/4 Bones
5×10^7
4×10^7
3×10^7
2×10^7
1×10^7
0
> 0.01
Ctrl
IgG1
Dara

Figure 3B.


CD45+CD33+ Cells/Spleen
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6×10^6
4×10^6
2×10^6
0
< 0.001
Ctrl
IgG1
Dara

Figure 3C.

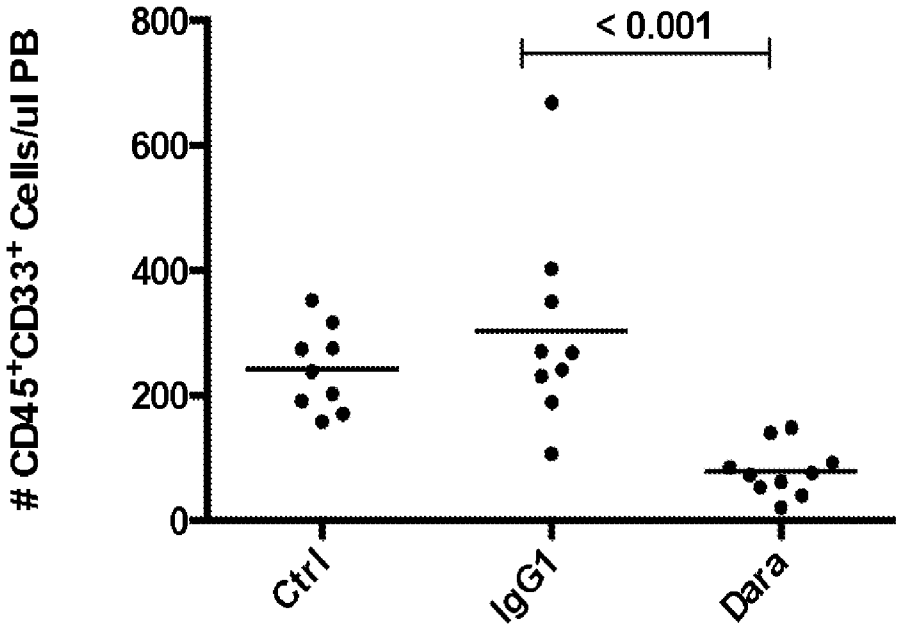


Figure 4A.

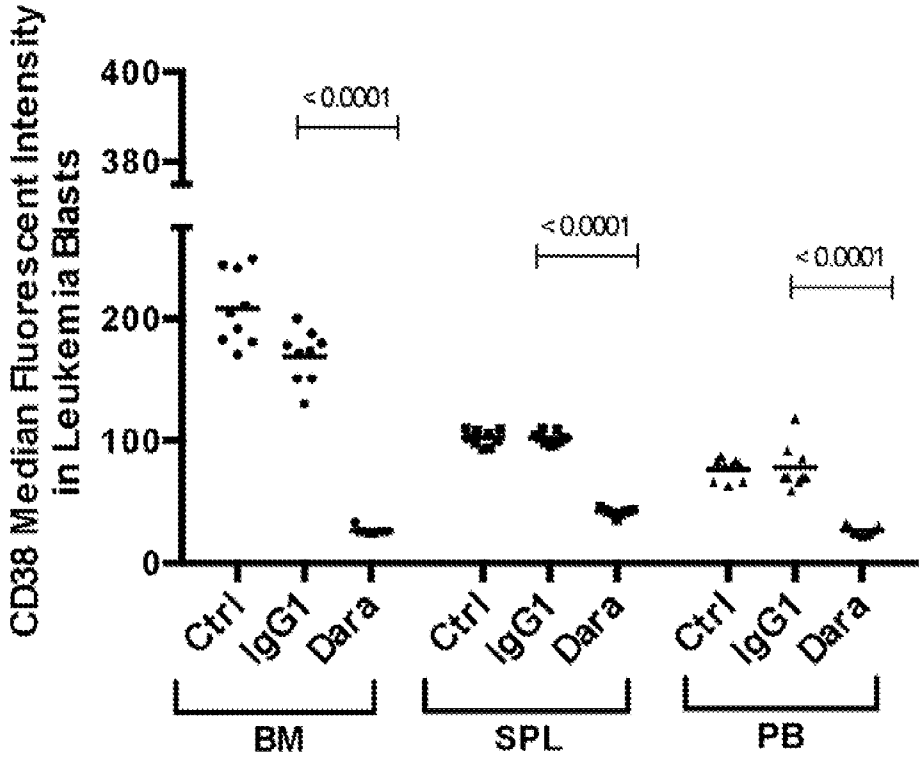

CD38 Median Fluorescent Intensity in Leukemia Blasts
400
380
200
100
0
< 0.0001
< 0.0001
< 0.0001
Ctrl
IgG1
Dara
Ctrl
IgG1
Dara
Ctrl
IgG1
Dara
BM
SPL
PB

Figure 4B.

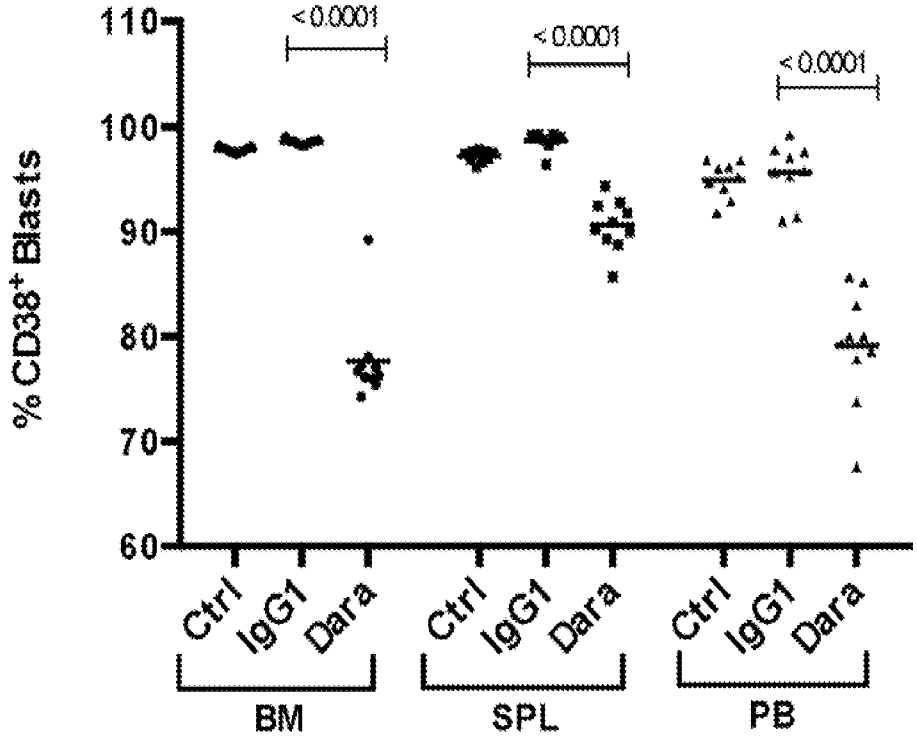

110
100
90
80
70
60
% CD38+ Blasts
< 0.0001
< 0.0001
< 0.0001
Ctrl
IgG1
Dara
Ctrl
IgG1
Dara
Ctrl
IgG1
Dara
BM
SPL
PB

Figure 5A.

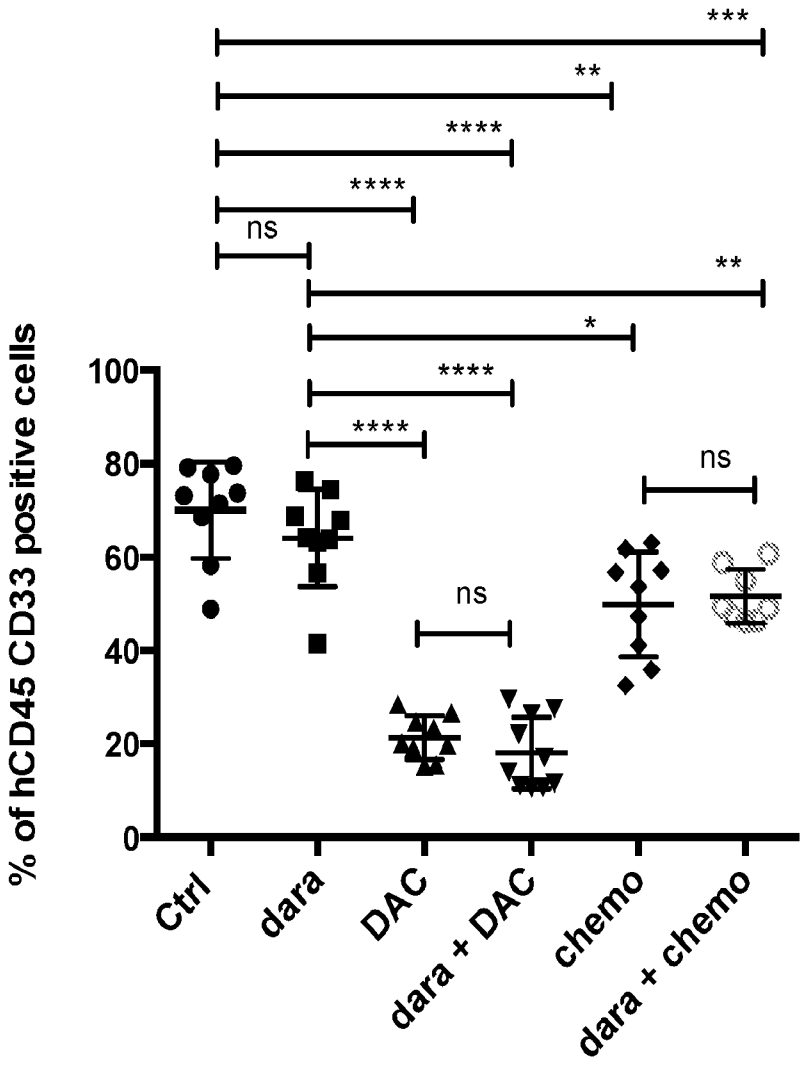

***
**
****
****
ns
**
*
****
****
ns
ns
100
80
60
40
20
0
% of hCD45 CD33 positive cells
Ctrl
dara
DAC
dara + DAC
chemo
dara + chemo

Figure 5B.

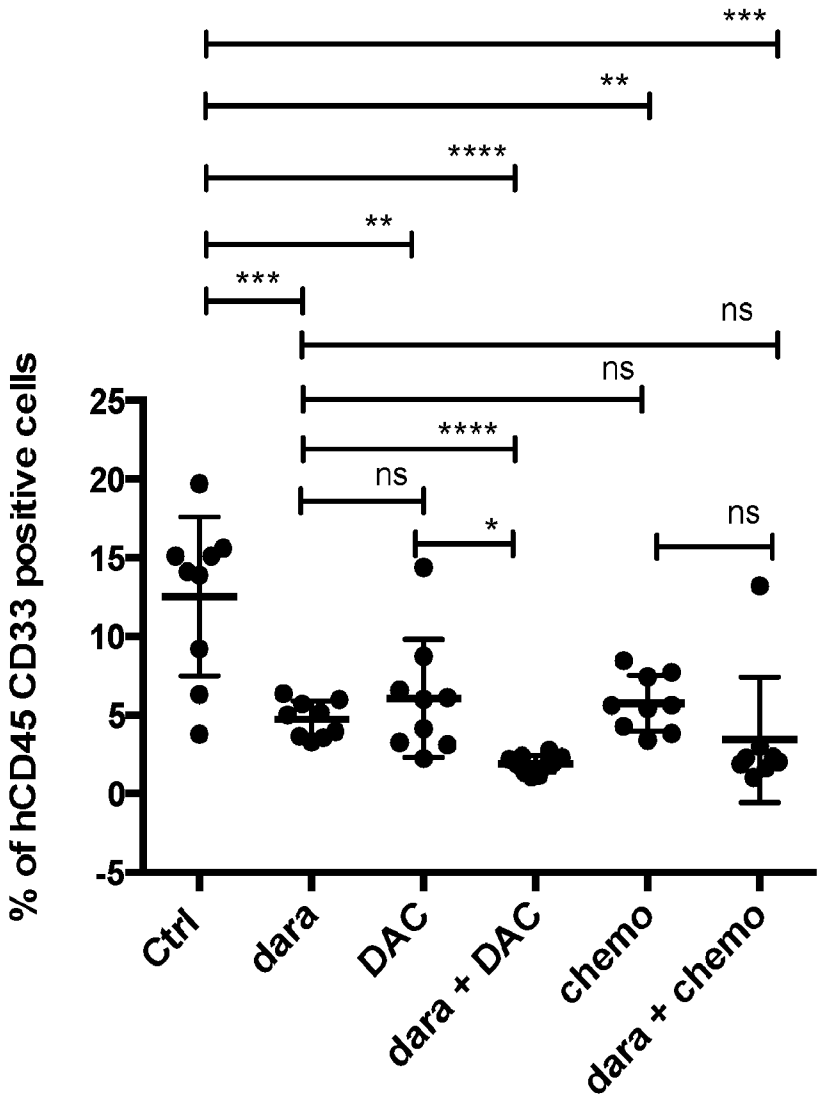

***
**
****
**
***
ns
ns
****
ns
*
ns
% of hCD45 CD33 positive cells
25
20
15
10
5
0
-5
Ctrl
dara
DAC
dara + DAC
chemo
dara + chemo

Figure 5C.

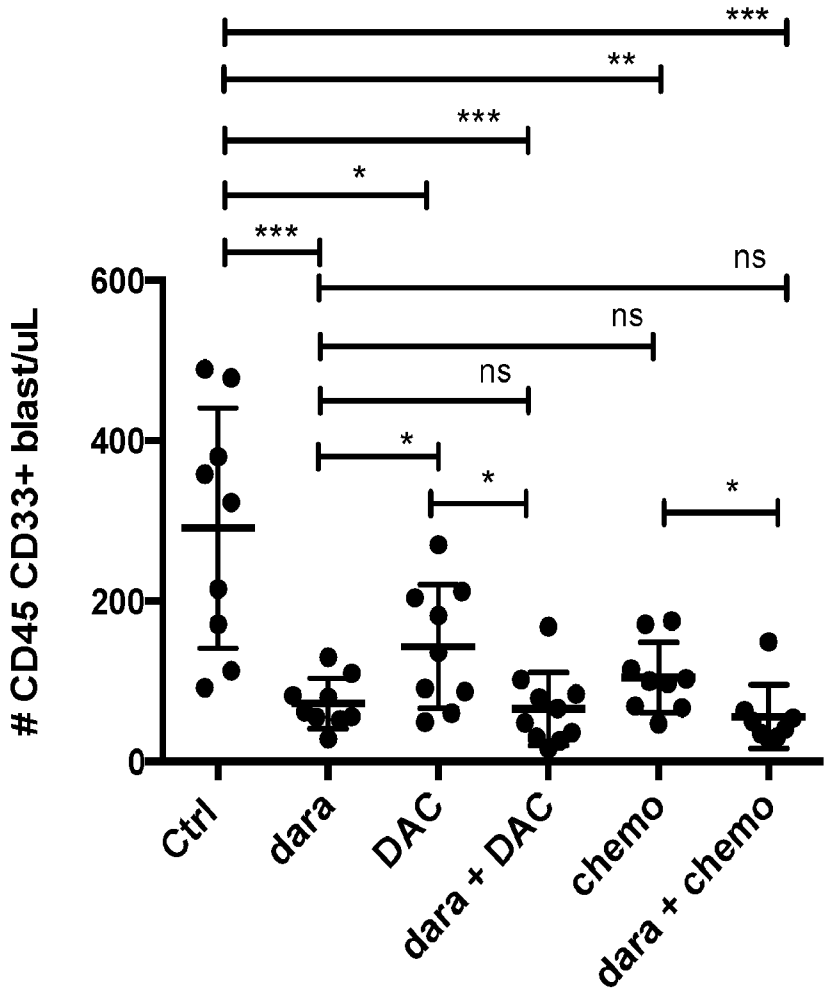

***
**
***
*
***
ns
ns
ns
*
*
*
600
400
200
0
CD45 CD33+ blast/uL
Ctrl
dara
DAC
dara + DAC
chemo
dara + chemo

Figure 6A.

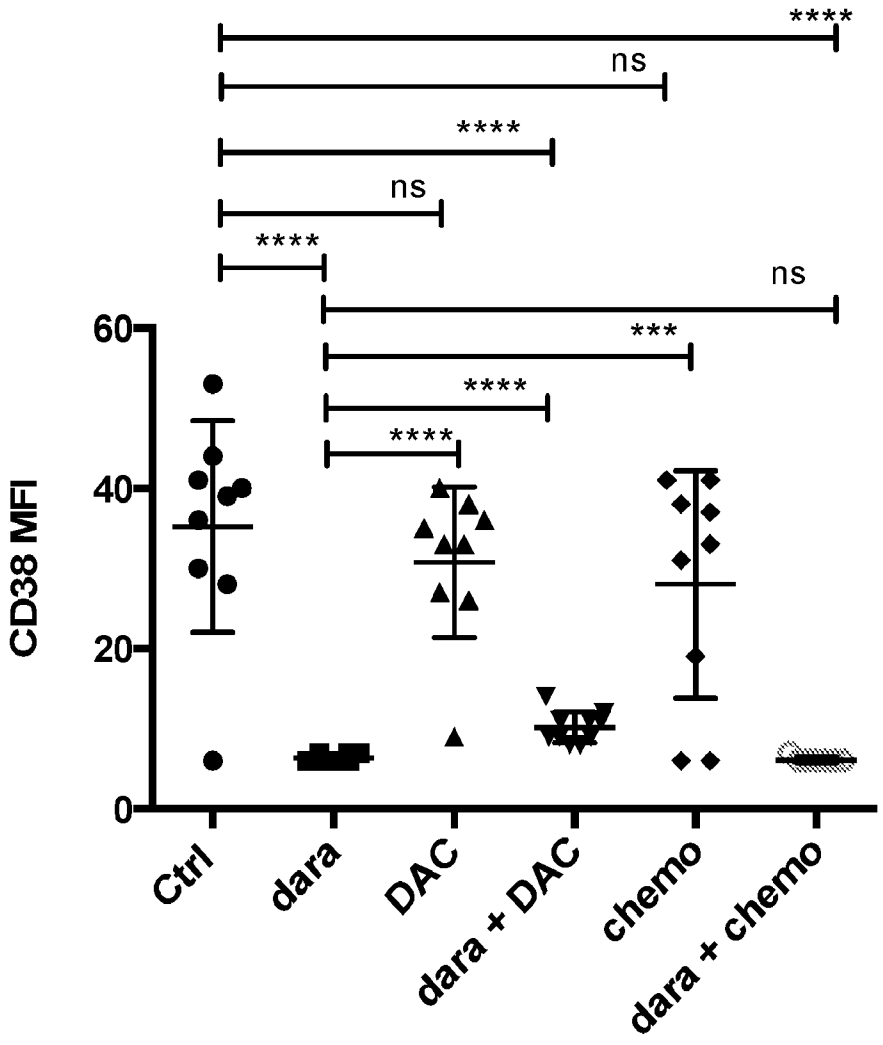

****
ns
****
ns
****
ns
***
****
****
60
40
20
0
CD38 MFI
Ctrl
dara
DAC
dara + DAC
chemo
dara + chemo

Figure 6B.

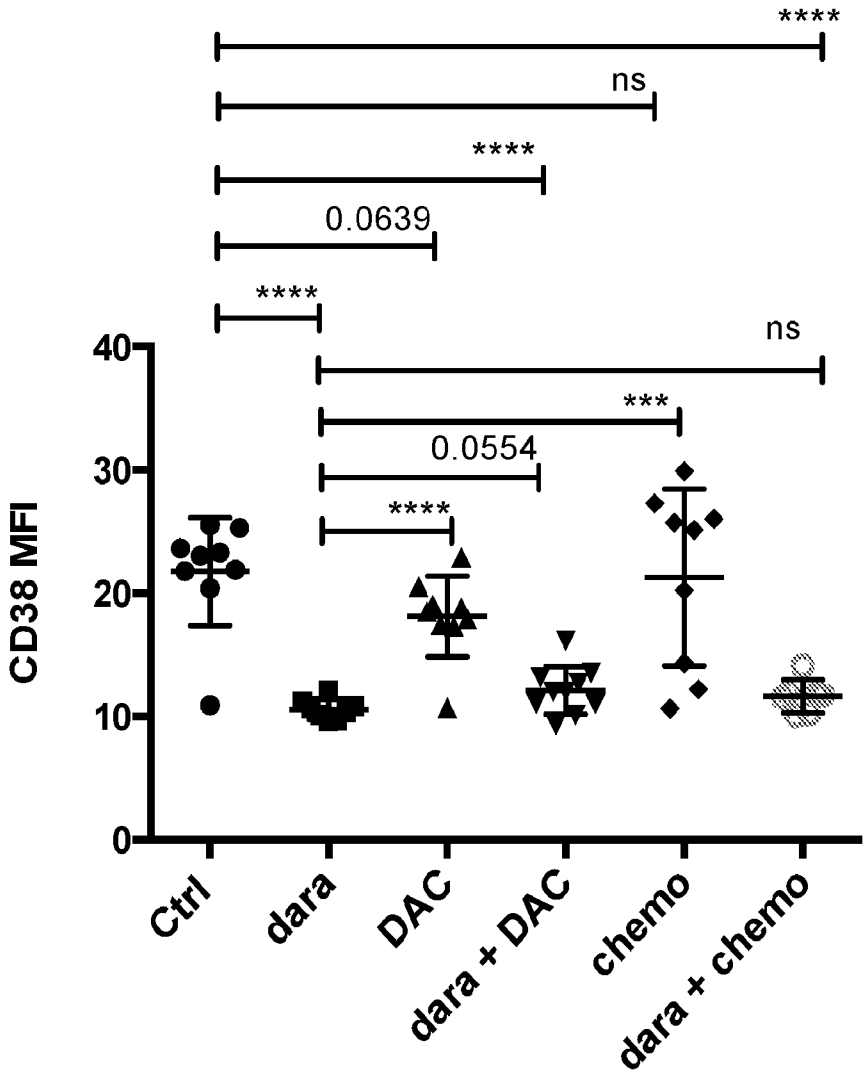
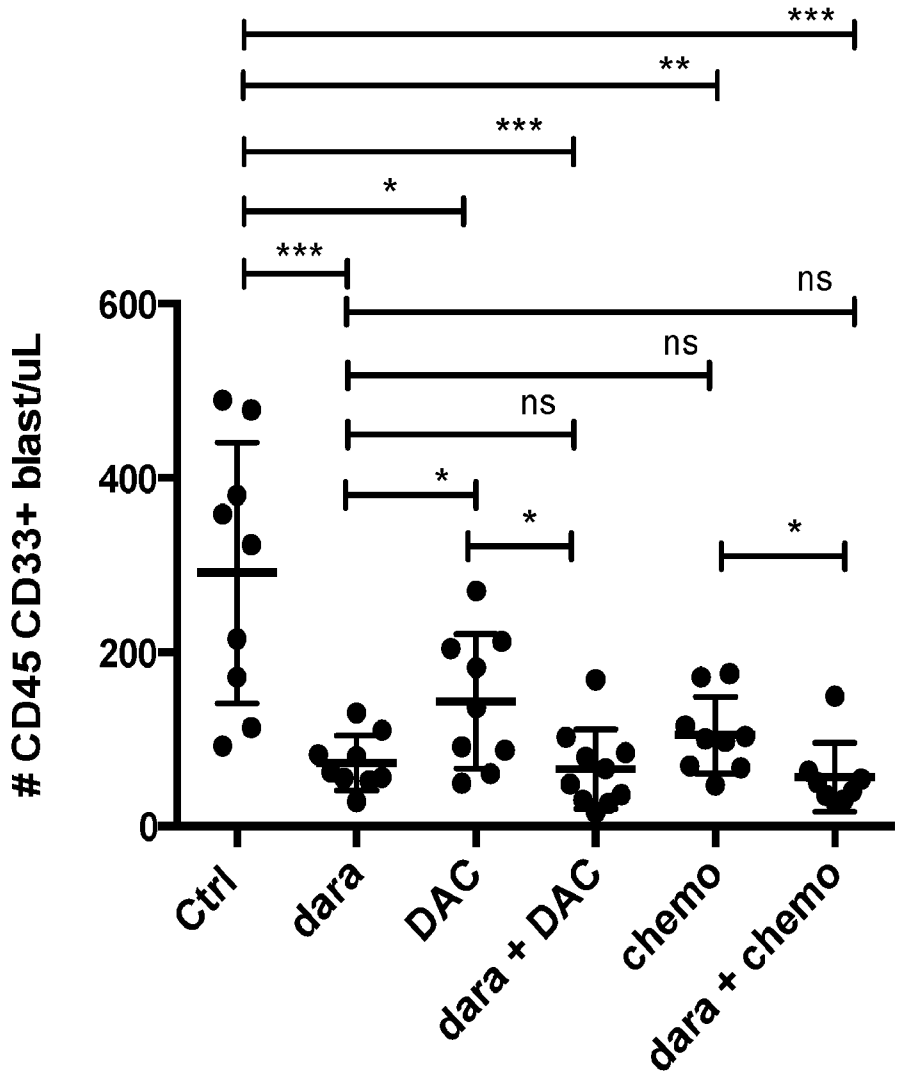

****
ns
****
0.0639
****
ns
***
0.0554
****
CD38 MFI
40
30
20
10
0
Ctrl
dara
DAC
dara + DAC
chemo
dara + chemo

Figure 6C.


***
**
***
*
***
ns
ns
ns
*
*
*
600
400
200
0
CD45 CD33+ blast/uL
Ctrl
dara
DAC
dara + DAC
chemo
dara + chemo

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US15/63371

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 16/28; A61K 39/395; A61P 35/02 (2016.01)
CPC - A61K 39/39558, 39/3955; C07K 16/2896
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): C07K 16/28, 16/18, 16/00; A61K 39/395, 39/00; A61P 35/02 (2016.01)
CPC: A61K 39/39558, 39/3955, 39/00; C07K 16/2896, 16/28, 16/18, 16/00, 2316/95, 2316/96

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/19.6, 19.3, 19.2, 1.1, 1; 424/130.1, 138.1, 139.1, 141.1, 143.1, 144.1, 152.1, 155.1; 530/387.1, 386, 380, 350

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Patseer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC, RU, AT, CH, TH, BR, PH); The Lens.org PatSeq; Pubmed/NCBI Blast; EBSCO; Google/Google Scholar/Google Patents: [SEQ ID NOs: 1-5], 'anti-CD38,' 'acute myeloid leukemia,' 'AML,' 'same epitope,' antibody, compete, binding, daratumumab, 'SKRNIQFSCKNIYR,' 'EKVQTLEAWVIHGG'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2006/099875 A1 (GENMAB A/S) September 28, 2006; page 8, lines 6-17; page 13, lines 22-26; page 20, lines 23-25; page 53, lines 6-15; page 168, lines 10-13; page 169, lines 11-13	1-3 --- 2, 3/1, 3/2
X --- Y	US 2010/0285004 A1 (TESAR, M et al.) November 11, 2010; paragraphs [0021], [0058], [0107]-[0109]	1 --- 2, 3/1, 3/2
A	US 2013/0209355 A1 (DE WEERS, M et al.) 15 August 2013; abstract	1-3
A	DEWEERS, M et al. Daratumumab, A Novel Therapeutic Human CD38 Monoclonal Antibody, Induces Killing of Multiple Myeloma and Other Hematological Tumors. J Immunol. 01 February 2011, Vol. 186, No. 3; pages 1840-1848, DOI. 10.4049/jimmunol.1003032.	1-3
P, Y	VAN BUEREN, JL et al. Direct In Vitro Comparison Of Daratumumab With Surrogate Analogs Of Anti-CD38 Antibodies. New Evidence. April 2015 [retrieved on 03 February 2016] Retrieved from the Internet: <URL: http://www.newevidence.com/oncology/direct-in-vitro-comparison-of-daratumumab-with-surrogate-analogs-of-anti-cd38-antibodies/>	1-3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 February 2016 (05.02.2016)	19 FEB 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US15/63371

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos :
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-26
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

(19)中华人民共和国国家知识产权局

(12)发明专利申请

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(43)申请公布日 2017.11.28

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(22)申请日 2015.12.02

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62/087,442 2014.12.04 US

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2017.08.04

(86)PCT国际申请的申请数据
PCT/US2015/063371 2015.12.02

(87)PCT国际申请的公布数据
WO2016/089960 EN 2016.06.09

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代理人 彭昶 黄希贵

(51)Int.Cl.
C07K 16/28(2006.01)
A61K 39/395(2006.01)
A61P 35/02(2006.01)

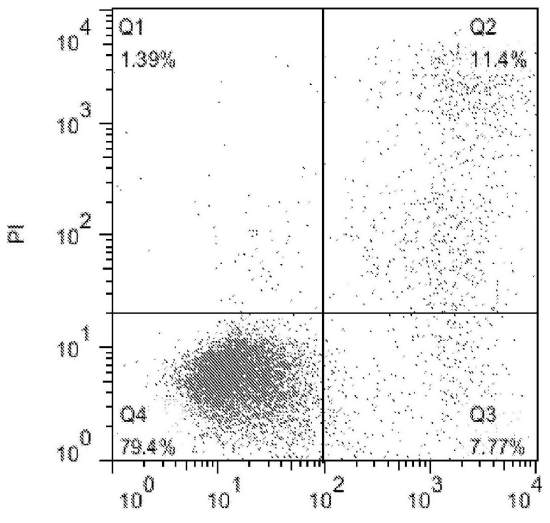
权利要求书2页 说明书31页
序列表16页 附图12页

(54)发明名称

用于治疗急性髓系白血病的抗CD38抗体

(57)摘要

本发明涉及采用抗CD38抗体来治疗急性髓系白血病的方法。



CN 107406506 A

1.一种治疗患有急性髓系白血病(AML)的受治疗者的方法,所述方法包括向对其有需要的受治疗者施用抗CD38抗体足以治疗AML的一段时间。

2.根据权利要求1所述的方法,其中所述抗CD38抗体与包含SEQ ID NO:4的重链可变区(VH)和SEQ ID NO:5的轻链可变区(VL)的抗体竞争结合SEQ ID NO:1的人CD38。

3.根据权利要求1或2所述的方法,其中所述抗CD38抗体结合于人CD38(SEQ ID NO:1)的区域SKRNIQFSCKNIYR(SEQ ID NO:2)和区域EKVQTLEAWVIHGG(SEQ ID NO:3)。

4.根据权利要求1-3中任一项所述的方法,其中所述抗CD38抗体通过细胞凋亡来诱导杀伤表达CD38的AML细胞。

5.根据权利要求1-4中任一项所述的方法,其中所述抗CD38抗体具有IgG1、IgG2、IgG3或IgG4同种型。

6.根据权利要求1-5中任一项所述的方法,其中所述抗CD38抗体包含分别为SEQ ID NO:6、7和8的重链互补决定区(HCDR)1(HCDR1)、2(HCDR2)和3(HCDR3)序列,以及分别为SEQ ID NO:9、10和11的轻链互补决定区(LCDR)1(LCDR1)、2(LCDR2)和3(LCDR3)序列。

7.根据权利要求6所述的方法,其中所述抗CD38抗体包含SEQ ID NO:4的重链可变区(VH)和SEQ ID NO:5的轻链可变区(VL)。

8.根据权利要求7所述的方法,其中所述抗CD38抗体包含SEQ ID NO:12的重链和SEQ ID NO:13的轻链。

9.根据权利要求1-5中任一项所述的方法,其中所述抗CD38抗体包含分别为SEQ ID NO:15和16的VH和VL。

10.根据权利要求1-5中任一项所述的方法,其中所述抗CD38抗体包含分别为SEQ ID NO:17和18的VH和VL。

11.根据权利要求1-5中任一项所述的方法,其中所述抗CD38抗体包含分别为SEQ ID NO:19和20的VH和VL。

12.根据权利要求1-5中任一项所述的方法,其中所述抗CD38抗体包含分别为SEQ ID NO:21和22的VH和VL。

13.根据权利要求1-12中任一项所述的方法,其中AML为伴有至少一种遗传异常的AML、伴有多系病变的AML、治疗相关性AML、未分化型AML、微成熟型AML、成熟型AML、急性髓单核细胞白血病、急性单核细胞白血病、急性红白血病、急性巨核细胞白血病、急性嗜碱性白血病、急性全髓纤维化或髓系肉瘤。

14.根据权利要求13所述的方法,其中所述至少一种遗传异常是染色体8和21之间的易位,染色体16中的易位或倒位,染色体15和17之间的易位,染色体11的改变,或者fms-相关的酪氨酸激酶3(FLT3)、核仁磷酸蛋白(NPM1)、异柠檬酸脱氢酶1(IDH1)、异柠檬酸脱氢酶2(IDH2)、DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)、CCAAT/增强子结合蛋白α(CEBPA)、U2小核RNA辅助因子1(U2AF1)、zeste 2多梳抑制复合物2亚基的增强子(EZH2)、染色体1A的结构维持(SMC1A)或染色体3的结构维持(SMC3)中的突变。

15.根据权利要求13所述的方法,其中所述至少一种遗传异常是IDH1中的易位t(8;21)(q22;q22)、倒位inv(16)(p13;q22)、易位t(16;16)(p13;q22)、易位t(15;17)(q22;q12)、突变FLT3-ITD、突变R132H或R100Q/R104V/F108L/R119Q/I130V或者IDH2中的突变R140Q或R172。

16.根据权利要求1-15中任一项所述的方法，其中AML是难治的或复发的。

17.根据权利要求1-16中任一项所述的方法，其中所述抗CD38抗体作为缓解诱导、缓解后或维持疗法来施用。

18.根据权利要求1-17中任一项所述的方法，其中所述抗CD38抗体与至少一种第二治疗剂联合施用。

19.根据权利要求18所述的方法，其中所述至少一种第二治疗剂为阿糖胞苷、佐柔比星、伊达比星、米托蒽醌、羟基脲、地西他滨、克拉屈滨、氟达拉滨、拓朴替康、依托泊苷6-硫鸟嘌呤、皮质类固醇、泼尼松、地塞米松、甲氨蝶呤、6-巯嘌呤、阿扎胞苷、三氧化二砷或全反式视黄酸。

20.根据权利要求18所述的方法，其中所述至少一种第二治疗剂为全反式视黄酸、阿糖胞苷、地西他滨或多柔比星。

21.根据权利要求17-20中任一项所述的方法，其中所述抗CD38抗体和所述至少一种第二治疗剂同时、依序或分开施用。

22.根据权利要求17-21中任一项所述的方法，其中所述至少一种第二治疗剂增加AML细胞上CD38的表面表达。

23.根据权利要求1-22中任一项所述的方法，其中所述受治疗者进一步经历放射疗法治疗或已经历放射疗法治疗。

24.根据权利要求1-23中任一项所述的方法，其中所述受治疗者正经受造血干细胞移植(HSCT)。

25.根据权利要求24所述的方法，其中所述HSCT是异源的、自体同源的或同基因的。

26.根据权利要求25所述的方法，其中所述HSCT包括来源于骨髓、血液或羊水的血液干细胞的移植。

用于治疗急性髓系白血病的抗CD38抗体

技术领域

[0001] 本发明涉及采用抗CD38抗体来治疗急性髓系白血病的方法。

背景技术

[0002] CD38是一种II型膜蛋白,具有ADP核糖基环化酶活性,分别催化NAD和NADP形成第二信使环ADP-核糖(cADPR)和烟酸腺嘌呤二核苷酸磷酸(NAADP)。CD38介导钙动员并调节细胞内NAD水平,并且研究表明在多种生理功能中具有作用(Funaro等人,J Immunology 145:2390-6,1990;Terhorst等人,Cell 771-80,1981;Guse等人,Nature 398:70-3,1999;Adriouch等人,14:1284-92,2012;Chiarugi等人,Nature Reviews 12:741-52,2012;Wei等人,WJBC 5:58-67,2014)。

[0003] 急性髓系白血病(AML)是一种异质性血液病,其特征在于骨髓、外周血和其它组织中的髓系母细胞的克隆扩充。尽管近来取得了进展,当前对AML的治疗仍未令人满意,5-年无复发生存率低于30%。

[0004] 因此,仍然需要有效的AML疗法。

发明内容

[0005] 本发明的一个实施方案是一种治疗患有急性髓系白血病(AML)的受治疗者的方法,该方法包括向对其有需要的受治疗者施用抗CD38抗体足以治疗AML的一段时间。

[0006] 本发明的一个实施方案是一种治疗患有急性髓系白血病(AML)的受治疗者的方法,该方法包括向对其有需要的受治疗者施用抗CD38抗体足以治疗AML的一段时间,该抗CD38抗体与包含SEQ ID NO:4的重链可变区(VH)和SEQ ID NO:5的轻链可变区(VL)的抗体竞争结合CD38。

附图说明

[0007] 图1A示出在NB-4 AML细胞系中不存在交联时达雷木单抗诱导的细胞凋亡。PI:碘化丙啶。

[0008] 图1B示出在NB-4 AML细胞系中存在交联时达雷木单抗诱导的细胞凋亡。PI:碘化丙啶。

[0009] 图2A示出达雷木单抗在来源于患者的异种移植(PDX)AML 3406模型中的疗效,其通过骨髓(BM)、脾(SPL)和外周血(PB)中白血病$CD45^+CD33^+$细胞的减少百分比(%)进行测量。Ctrl:未治疗;IgG1:同种型对照;Dara:达雷木单抗。P值在图中示出(同种型对照对达雷木单抗)。

[0010] 图2B示出达雷木单抗在来源于患者的异种移植(PDX)AML 7577模型中的疗效,其通过骨髓(BM)、脾(SPL)和外周血(PB)中白血病$CD45^+CD33^+$细胞的减少百分比(%)进行测量。Ctrl:未治疗;IgG1:同种型对照;Dara:达雷木单抗。ns:不显著。***$p<0.001$

[0011] 图2C示出达雷木单抗在来源于患者的异种移植(PDX)AML 8096模型中的疗效,其

通过骨髓(BM)、脾(SPL)和外周血(PB)中白血病$CD45^+CD33^+$细胞的减少百分比(%)进行评估。Ctrl:未治疗;IgG1:同种型对照;Dara:达雷木单抗。ns:不显著。*p<0.05

[0012] 图3A示出达雷木单抗在来源于患者的异种移植(PDX)AML 3406模型中的疗效,其通过骨髓中总白血病负荷(每四块骨的$CD45^+CD33^+$细胞数)的减少进行评估。Ctrl:未治疗;IgG1:同种型对照;Dara:达雷木单抗。Ctrl和Dara之间的骨髓中白血病负荷无显著差异(p>0.01)。示出同种型对照对达雷木单抗治疗组之间的P值。

[0013] 图3B示出达雷木单抗在来源于患者的异种移植(PDX)AML 3406模型中的疗效,其通过脾中总白血病负荷(每个脾的$CD45^+CD33^+$细胞数)的减少进行评估。Ctrl:未治疗;IgG1:同种型对照;Dara:达雷木单抗。示出同种型对照对达雷木单抗治疗组之间的P值。

[0014] 图3C示出达雷木单抗在来源于患者的异种移植(PDX)AML 3406模型中的疗效,其通过外周血中总白血病负荷(每μl血液的$CD45^+CD33^+$细胞数)的减少进行评估。Ctrl:未治疗;IgG1:同种型对照;Dara:达雷木单抗。示出同种型对照对达雷木单抗治疗组之间的P值。

[0015] 图4A示出在骨髓(BM)、脾(SPL)和外周血(PB)中用达雷木单抗治疗5周后,在来源于患者的异种移植(PDX)AML 3406模型中的达雷木单抗诱导的表面CD38表达的下调。Ctrl:未治疗;IgG1:同种型对照;Dara:达雷木单抗。P值如图所示(同种型对照对达雷木单抗)。

[0016] 图4B示出在骨髓(BM)、脾(SPL)和外周血(PB)中用达雷木单抗治疗5周后,在来源于患者的异种移植(PDX)AML 3406模型中的达雷木单抗诱导的CD38-阳性白血病母细胞百分比的减少。Ctrl:未治疗;IgG1:同种型对照;Dara:达雷木单抗。示出同种型对照对达雷木单抗治疗组之间的P值。

[0017] 图5A示出达雷木单抗(dara)单独使用或与达克金(DAC)或阿糖胞苷和多柔比星(chemo)联合使用时对减少来源于患者的异种移植(PDX)3406模型中的骨髓白血病负荷的疗效。白血病负荷用$CD45^+CD33^+$细胞%进行评估。Ctrl:同种型对照。*p<0.05;**p<0.01;***p<.001。ns:不显著。

[0018] 图5B示出达雷木单抗(dara)单独使用或与达克金(DAC)或阿糖胞苷和多柔比星(chemo)联合使用时对减少来源于患者的异种移植(PDX)3406模型中的脾白血病负荷的疗效。白血病负荷用$CD45^+CD33^+$细胞%进行评估。Ctrl:同种型对照。*p<0.05;**p<0.01;***p<0.001。ns:不显著。

[0019] 图5C示出达雷木单抗(dara)单独使用或与达克金(DAC)或阿糖胞苷和多柔比星(chemo)联合使用时对减少来源于患者的异种移植(PDX)模型中的外周血白血病负荷的疗效。白血病负荷用$CD45^+CD33^+$细胞%进行评估。Ctrl:同种型对照。*p<0.05;**p<0.01;***p<0.001。ns:不显著。

[0020] 图6A示出达雷木单抗(dara)单独使用或与达克金(DAC)或阿糖胞苷和多柔比星(chemo)联合使用时对来源于患者的异种移植(PDX)3406模型中的$CD45^+CD33^+$AML骨髓母细胞上的CD38表达的影响。白血病负荷用$CD45^+CD33^+$细胞%进行评估。Ctrl:同种型对照。*p<0.05;**p<0.01;***p<0.001。ns:不显著。MFI:平均荧光强度。

[0021] 图6B示出达雷木单抗(dara)单独使用或与达克金(DAC)或阿糖胞苷和多柔比星(chemo)联合使用时对来源于患者的异种移植(PDX)3406模型中的$CD45^+CD33^+$AML脾母细胞上的CD38表达的影响。白血病负荷用$CD45^+CD33^+$细胞%进行评估。Ctrl:同种型对照。*p<0.05;**p<0.01;***p<0.001。ns:不显著。

[0022] 图6C示出达雷木单抗(dara)单独使用或与达克金(DAC)或阿糖胞苷和多柔比星(chemo)联合使用时对来源于患者的异种移植(PDX)3406模型中的$CD45^+CD33^+$AML外周血母细胞上的CD38表达的影响。白血病负荷用$CD45^+CD33^+$细胞%进行评估。Ctrl:同种型对照。*p<0.05;**p<0.01;***p<0.001。ns:不显著。

具体实施方式

[0023] "CD38"是指人CD38蛋白(别名:ADP核糖基环化酶1、cADPr水解酶1、环ADP核糖水解酶1)。人CD38具有SEQ ID NO:1所示的氨基酸序列。

[0024] 如本文所用,"抗体"含义广泛且包括免疫球蛋白分子,其包括单克隆抗体,包括鼠、人、人适应性、人源化和嵌合单克隆抗体;抗体片段;双特异性或多特异性抗体;二聚体、四聚体或多聚体抗体;以及单链抗体。

[0025] 免疫球蛋白可根据重链恒定域氨基酸序列被指定为五种主要种类,即IgA、IgD、IgE、IgG和IgM。IgA和IgG进一步亚分类为同种型IgA_1、IgA_2、IgG_1、IgG_2、IgG_3和IgG_4。任何脊椎动物物种的抗体轻链可基于其恒定域的氨基酸序列被指定为两种完全不同的类型中的一种,即κ(κ)和λ(λ)。

[0026] "抗体片段"是指免疫球蛋白分子的一部分,其保留重链和/或轻链抗原结合位点,诸如重链互补决定区(HCDR)1、2和3,轻链互补决定区(LCDR)1、2和3,重链可变区(VH)或轻链可变区(VL)。抗体片段包括:Fab片段,即由VL、VH、CL和CHI域组成的单价片段;$F(ab)_2$片段,即包含在铰链区通过二硫键连接的两个Fab片段的二价片段;由VH和CHI域组成的Fd片段;由抗体单臂的VL和VH域组成的Fv片段;域抗体(dAb)片段(Ward等人,(1989)Nature 341:544-546),其由VH域组成。VH域和VL域可经工程化并经由合成接头连接在一起以形成各种类型的单链抗体设计,其中在VH域和VL域由单独的单链抗体构建体表达的情况下,VH/VL域在分子内或分子间配对,以形成单价抗原结合位点,诸如单链Fv(scFv)或双链抗体;例如在PCT国际公布WO1998/44001、WO1988/01649、WO1994/13804和WO1992/01047中所描述的。使用本领域的技术人员熟知的技术获得这些抗体片段,并筛选出效用方式与全长抗体相同的片段。

[0027] 短语"分离抗体"是指基本上不含具有不同抗原特异性的其它抗体的抗体或抗体片段(例如,特异性结合CD38的分离抗体基本上不含特异性结合人CD38之外的抗原的抗体)。然而,特异性结合CD38的分离抗体可能对其它抗原具有交叉反应性,诸如人CD38的直系同源抗原,诸如食蟹猕猴(Macaca fascicularis)(食蟹猴)CD38。此外,分离抗体可基本上不含其它细胞物质和/或化学物质。

[0028] 抗体可变区由被三个"抗原结合位点"中断的"框架"区组成。使用多个术语来定义抗原结合位点:三个在VH(HCDR1、HCDR2、HCDR3)中且三个在VL(LCDR1、LCDR2、LCDR3)中的互补决定区(CDR)基于序列变异性(Wu和Kabat,J Exp Med 132:211-50,1970;Kabat等人,Sequences of Proteins of Immunological Interest,第5版,Public Health Service,National Institutes of Health,Bethesda,Md.,1991),三个在VH(H1、H2、H3)中且三个在VL(L1、L2、L3)中的"超变区"、"HVR"或"HV",是指抗体可变域中的在结构上超变的区域,如Chothia和Lesk所定义(Chothia and Lesk Mol Biol 196:901-17,1987)。其它术语包括"IMGT-CDR"(Lefranc等人,Dev Comparat Immunol 27:55-77,2003)和"特异性决定残基用

途”(SDRU)(Almagro,Mol Recognit 17:132-43,2004)。国际ImMunoGeneTics(IMGT)数据库(http://www_imgt_org)提供了抗原结合位点的标准化编号和定义。CDR、HV与IMGT划分之间的对应性描述于Lefranc等人,Dev Comparat Immunol 27:55-77,2003。

[0029] 如本文所用,“Chothia残基”是抗体VL和VH残基,其根据Al-Lazikani来编号(Al-Lazikani等人,J Mol Biol 273:927-48,1997)。

[0030] “框架”或“框架序列”是可变区的除了被定义为抗原结合位点的那些序列之外的其余序列。因为抗原结合位点可以由如上所述的各种术语定义,所以框架的精确氨基酸序列取决于如何定义抗原结合位点。

[0031] “人源化抗体”是指其中抗原结合位点来源于非人物种且可变区框架来源于人免疫球蛋白序列的抗体。人源化抗体在框架区中可包含置换,使得该框架可能不是表达的人免疫球蛋白或种系基因序列的精确拷贝。

[0032] “人适应性”抗体或“人框架适应性(HFA)”抗体是指根据美国专利公布US2009/0118127中所描述的方法适应化的人源化抗体。通过如下方式将人适应性抗体人源化:基于CDR1和CDR2环及一部分轻链CDR3环的最大CDR与FR相似性、长度相容性和序列相似性,来选择受体人框架。

[0033] “人抗体”是指具有重链可变区和轻链可变区的抗体,其中框架和抗原结合位点均来源于人起源的序列。如果所述抗体包含恒定区,则该恒定区也来源于人起源的序列。

[0034] 人抗体包含“来源于”人起源序列的重链可变区或轻链可变区,其中抗体的可变区来源于使用人种系免疫球蛋白或重排免疫球蛋白基因的系统。此类系统包括在噬菌体上展示的人免疫球蛋白基因文库,以及转基因非人动物,诸如携带如本文所述的人免疫球蛋白基因座的小鼠。在与人种系或重排免疫球蛋白序列进行比较时,“人抗体”可包含由于例如天然存在的体细胞突变或者在框架或抗原结合位点中特意引入置换所引起的氨基酸差异。通常,人抗体的氨基酸序列与由人种系或重排免疫球蛋白基因编码的氨基酸序列具有至少约80%、81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%的同一性。在一些情况下,“人抗体”可包含由人框架序列分析得到的共有框架序列(例如Knappik等人,J Mol Biol 296:57-86,2000中所述);或结合到展示在噬菌体上的人免疫球蛋白基因文库中的合成HCDR3(例如Shi等人,J Mol Biol 397:385-96,2010和国际专利公布WO2009/085462中所述)。人抗体的定义中不包括抗原结合位点来源于非人物种的抗体。

[0035] 分离的人源化抗体可以是合成的。人抗体可使用诸如噬菌体展示结合合成CDR和/或合成框架的系统产生,或可经受体外诱变以改善抗体特性。

[0036] 如本文所用,“重组抗体”包括通过重组方法制备、表达、创建或分离的所有抗体,诸如:从动物(例如小鼠或大鼠)分离的抗体,即人免疫球蛋白基因的转基因或转染色体,或从其制备的杂交瘤分离的抗体(在下文中进一步描述),从经转化以表达抗体的宿主细胞分离的抗体,从重组的、组合的抗体文库分离的抗体,以及通过涉及将人免疫球蛋白基因序列与其它DNA序列剪接在一起的任何其它方法制备、表达、创建或分离的抗体,或使用例如Fab臂交换体外生成的抗体,诸如双特异性抗体。

[0037] 如本文所用,“单克隆抗体”是指单分子组合物的抗体分子的制剂。单克隆抗体组合物显示出对于特定表位的单一结合特异性和亲和力,或就双特异性单克隆抗体而言,显

示出对于两种不同表位的双重结合特异性。

[0038] 如本文所用，“表位”意指抗原的与抗体特异性结合的部分。表位通常由部分(moiety)(诸如氨基酸或多糖侧链)的化学活性(诸如，极性、非极性或疏水性)表面基团构成，并且可以具有特定三维结构特征以及特定电荷特征。表位可以由形成构象空间单元的连续或不连续氨基酸组成。对于不连续表位，来自抗原的线性序列的不同部分的氨基酸因蛋白质分子的折叠而在三维空间上靠近。

[0039] 如本文所用，“变体”是指因一处或多处修饰(例如置换、插入或缺失)而不同于参考多肽或参考多核苷酸的多肽或多核苷酸。

[0040] “协同”、“协同作用”或“协同的”意指超过组合的预期加性效应。

[0041] 如本文所用，术语“与……联合使用”意指两种或更多种治疗剂可以在混合物中一起、作为单一药剂同时地或作为单一药剂以任何顺序依次地施用于受治疗者。

[0042] “治疗”或“诊疗”是指治疗剂治疗，其中治疗对象将减慢(减轻)非期望的生理改变或疾病诸如肿瘤或肿瘤细胞的发展、扩充或扩散，或者用于在治疗期间提供有益或期望的临床结果。有益或期望的临床结果包括症状的减轻、疾病程度的减弱、疾病的稳定(即，未恶化)状态、疾病进展的延迟或减慢、疾病状态的改善或缓和，以及缓解(不论是部分缓解还是完全缓解)，不论是可检测的还是不可检测的。“治疗”也可意指与受治疗者未接受治疗时的预期生存期相比延长生存期。需要治疗的那些受治疗者包括已经患有非期望的生理改变或疾病的那些受治疗者、以及倾向于患有生理改变或疾病的那些受治疗者。

[0043] “抑制生长”(例如，涉及细胞，诸如肿瘤细胞)是指与本领域技术人员熟知的适当对照条件下生长的相同细胞的生长相比，在与治疗剂或者治疗剂或药物的组合接触时体外或体内细胞生长的可测量下降。体外或体内细胞生长的抑制可为至少约10％、20％、30％、40％、50％、60％、70％、80％、90％、99％或100％。细胞生长的抑制可通过多种机制发生，例如通过抗体依赖性细胞介导的细胞毒性(ADCC)、抗体依赖性细胞吞噬作用(ADCP)、补体依赖性细胞毒性(CDC)、细胞凋亡、坏死、CD38酶活性的抑制、或通过细胞增殖的抑制。

[0044] “治疗有效量”指在所需剂量和时间段有效实现所需治疗结果的量。治疗有效量可根据一些因素而变化，诸如个体的疾病状态、年龄、性别和重量，以及治疗剂或治疗剂组合在个体中引发所需应答的能力。有效治疗剂或治疗剂组合的示例性指标包括，例如：患者健康状况的改善，肿瘤负荷的减少，肿瘤生长的遏止或减慢，和/或癌细胞没有向身体其它部位转移的情况。

[0045] 本文所述的本发明的一个实施方案，并且其处在下文所列所有带编号的实施方案的一些实施方案中，是治疗患有急性髓系白血病(AML)的受治疗者的方法，该方法包括向对其有需要的受治疗者施用抗CD38抗体足以治疗AML的一段时间。

[0046] 本文所述的本发明的另一个实施方案，并且其处在下文所列所有带编号的实施方案的一些实施方案中，是治疗患有急性髓系白血病(AML)的受治疗者的方法，该方法包括向对其有需要的受治疗者施用抗CD38抗体足以治疗AML的一段时间，该抗CD38抗体与包含SEQ ID NO:4的重链可变区(VH)和SEQ ID NO:5的轻链可变区(VL)的抗体竞争结合CD38。

[0047] 本文所述的本发明的另一个实施方案，并且其处在下文所列所有带编号的实施方案的一些实施方案中，是治疗患有急性髓系白血病(AML)的受治疗者的方法，该方法包括向对其有需要的受治疗者施用抗CD38抗体足以治疗AML的一段时间，该抗CD38抗体结合于人

CD38(SEQ ID NO:1)的区域SKRNIQFSCKNIYR(SEQ ID NO:2)和区域EKVQTLEAWVIHGG(SEQ ID NO:3)。

[0048] 抗CD38抗体结合于人CD38(SEQ ID NO:1)的区域SKRNIQFSCKNIYR(SEQ ID NO:2)和区域EKVQTLEAWVIHGG(SEQ ID NO:3),此时抗体结合至少1、2、3、4、5、6、7、8、9、10、11、12、13或14个残基,它们位于SEQ ID NO:2和SEQ ID NO:3内。在本文所公开的一些实施方案中(包括在下文所列带编号的实施方案中),抗CD38抗体结合人CD38(SEQ ID NO:1)的区域SKRNIQFSCKNIYR(SEQ ID NO:2)中的至少一个氨基酸以及区域EKVQTLEAWVIHGG(SEQ ID NO:3)中的至少一个氨基酸。在本文所公开的一些实施方案中(包括在下文所列带编号的实施方案中),抗CD38抗体结合人CD38(SEQ ID NO:1)的区域SKRNIQFSCKNIYR(SEQ ID NO:2)中的至少两个氨基酸以及区域EKVQTLEAWVIHGG(SEQ ID NO:3)中的至少两个氨基酸。在本文所公开的一些实施方案中(包括在下文所列带编号的实施方案中),抗CD38抗体结合人CD38(SEQ ID NO:1)的区域SKRNIQFSCKNIYR(SEQ ID NO:2)中的至少三个氨基酸以及区域EKVQTLEAWVIHGG(SEQ ID NO:3)中的至少三个氨基酸。在本文所公开的一些实施方案中(包括在下文所列带编号的实施方案中),抗CD38抗体结合人CD38(SEQ ID NO:1)的区域SKRNIQFSCKNIYR(SEQ ID NO:2)中的至少残基KRN以及区域EKVQTLEAWVIHGG(SEQ ID NO:3)中的至少残基VQLT(SEQ ID NO:14)。

[0049] 结合于人CD38(SEQ ID NO:1)的区域SKRNIQFSCKNIYR(SEQ ID NO:2)以及区域EKVQTLEAWVIHGG(SEQ ID NO:3)或最小限度地结合于如上所示的残基KRN和VQLT(SEQ ID NO:14)的示例性抗体是达雷木单抗(参见国际专利公布WO2006/0998647)。达雷木单抗包含分别以SEQ ID NO:4和5示出的VH和VL氨基酸序列,分别为SEQ ID NO:6、7和8的重链CDR HCDR1、HCDR2和HCDR3,以及分别为SEQ ID NO:9、10和11的轻链CDR LCDR1、LCDR2和LCDR3,并且具有IgG1/κ亚型。达雷木单抗重链氨基酸序列以SEQ ID NO:12示出,并且轻链氨基酸序列以SEQ ID NO:13示出。

[0050] 本文所述的本发明的另一个实施方案,并且其处在下文所列所有带编号的实施方案的一些实施方案中,是治疗患有急性髓系白血病(AML)的受治疗者的方法,该方法包括向对其有需要的受治疗者施用抗CD38抗体足以治疗AML的一段时间,该抗CD38抗体包含分别为SEQ ID NO:4和5的重链可变区(VH)和轻链可变区(VL)。

[0051] 本文所述的本发明的另一个实施方案,并且其处在下文所列所有带编号的实施方案的一些实施方案中,是治疗患有急性髓系白血病(AML)的受治疗者的方法,该方法包括向对其有需要的受治疗者施用抗CD38抗体足以治疗AML的一段时间,该抗CD38抗体包含分别为SEQ ID NO:6、7和8的重链CDR HCDR1、HCDR2和HCDR3,以及分别为SEQ ID NO:9、10和11的轻链CDR LCDR1、LCDR2和LCDR3。

[0052] SEQ ID NO:1

[0053] MANCEFSPVSGDKPCCRLSRRAQLCLGVSILVLILVVVLAVVVPRWRQQWSGPGTTKRFPETVLARCVKYTEIHPEMRHVDCQSVWDAFKGAFISKHPCNITEEDYQPLMKLGTQTVPCNKILLWSRIKDLAHQFTQVQRDMFTLEDTLLGYLADDLTWCGEFNTSKINYQSCPDWRKDCSNNPVSVFWKTVSRRFAEAACDVVHVMLNGSRSKIFDKNSTFGSVEVHNLQPEKVQTLEAWVIHGGREDSRDLCQDPTIKELESIISKRNIQFSCKNIYRPDKFLQCVKNPEDSSCTSEI

[0054] SEQ ID NO:2

[0055] SKRNIQFSCKNIYR

[0056] SEQ ID NO:3

[0057] EKVQTLEAWVIHGG

[0058] SEQ ID NO:4

[0059] EVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMSWVRQAPGKGLEWVSA

[0060] ISGSGGGTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYFCAKDK

[0061] ILWFGEPVFDYWGQGTLVTVSS

[0062] SEQ ID NO:5

[0063] EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGT DFTLTISSLEPEDFAVYYCQQRSNWPPTFGQGTKVEIK

[0064] SEQ ID NO:6

[0065] SFAMS

[0066] SEQ ID NO:7

[0067] AISGSGGGTYYADSVKG

[0068] SEQ ID NO:8

[0069] DKILWFGEPVFDY

[0070] SEQ ID NO:9

[0071] RASQSVSSYLA

[0072] SEQ ID NO:10

[0073] DASNRAT

[0074] SEQ ID NO:11

[0075] QQRSNWPPTF

[0076] SEQ ID NO:12

[0077] EVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMSWVRQAPGKGLEWVSAISGSGGGTYYADSVKGRFT ISRDNSKNTLYLQMNSLRAEDTAVYFCAKDKILWFGEPVFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0078] SEQ ID NO:13

[0079] EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGT DFTLTISSLEPEDFAVYYCQQRSNWPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0080] SEQ ID NO:14

[0081] VQLT

[0082] 可使用熟知的体外方法评估抗体与具有SEQ ID NO:4的VH和SEQ ID NO:5的VL的达雷木单抗对于结合CD38的竞争。在示例性方法中，重组表达CD38的CHO细胞可与未标记的达雷木单抗一起在4℃下温育15分钟，然后与过量的荧光标记的测试抗体一起在4℃下温育45分钟。在PBS/BSA中洗涤后，可使用标准方法通过流式细胞术测量荧光。在另一示例性方法中，人CD38的细胞外部分可包被在ELISA板的表面上。可在约15分钟内加入过量的未标记

达雷木单抗,随后可加入生物素酰化测试抗体。在PBS/吐温中洗涤后,可使用辣根过氧化物酶(HRP)缀合的链霉亲和素检测生物素酰化测试抗体的结合,并使用标准方法检测信号。显而易见的是,在竞争测定法中,达雷木单抗可带有标记,而测试抗体不带标记。测试抗体与达雷木单抗竞争,此时达雷木单抗抑制测试抗体的结合或测试抗体抑制达雷木单抗的结合达20%、30%、40%、50%、60%、70%、80%、85%、90%、95%或100%。可使用已知方法,通过例如肽作图或氢/氘保护测定法,或通过晶体结构测定,来进一步限定测试抗体的表位。

[0083] 在CD38上结合的区域与达雷木单抗相同的抗体可例如通过如下方式生成:使用标准方法并如本文所述的那样,利用具有以SEQ ID NO:2和3示出的氨基酸序列的肽来免疫小鼠。可例如通过如下方式进一步评估抗体:使用熟知的体外方法并如本文所述的那样,测定达雷木单抗与测试抗体两者对于结合CD38的竞争。

[0084] 其它示例性的抗CD38抗体可用于本文所述的本发明的任何实施方案以及下文所列所有带编号的实施方案的一些实施方案中,它们是:

[0085] 包含分别为SEQ ID NO:15和16的VH和VL序列的mAb003,并且其描述于美国专利7,829,693中。mAb003的VH和VL可表达为IgG1/κ。

[0086] SEQ ID NO:15

[0087] QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPGQGLEWMGRVIPFLGIANSAQKFQGRVTITADKSTSTAY

[0088] MDLSSLRSEDTAVYYCARDDIAALGPFDYWGQGTLVTVSSAS

[0089] SEQ ID NO:16

[0090] DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPRTFGQGTKVEIK;

[0091] 包含分别为SEQ ID NO:17和18的VH和VL序列的mAb024,其描述于美国专利7,829,693中。mAb024的VH和VL可表达为IgG1/κ。

[0092] SEQ ID NO:17

[0093] EVQLVQSGAEVKKPGESLKISCKGSGYSFSNYWIGWVRQMPGKGLEWMGIIYPHDSDARYSPSFQGQVTFSADKSISTAYLQWSSLKASDTAMYYCARHVGWGSRYWYFDLWGRGTLVTVSS

[0094] SEQ ID NO:18

[0095] EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPTFGQGTKVEIK;

[0096] 包含分别为SEQ ID NO:19和20的VH和VL序列的MOR-202(MOR-03087),其描述于美国专利8,088,896中。MOR-202的VH和VL可表达为IgG1/κ。

[0097] SEQ ID NO:19

[0098] QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYMNWVRQAPGKGLEWVSGISGDPSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDLPLVYTGFAYWGQGTLVTVSS

[0099] SEQ ID NO:20

[0100] DIELTQPPSVSVAPGQTARISCSGDNLRHYYVYWYQQKPGQAPVLVIYGDSKRPSGIPERFSGSNSGNTATLTISGT

QAEDEADYYCQTYTGGASLVFGGGTKLTVLGQ;

[0101] Isatuximab;其包含分别为SEQ ID NO:21和22的VH和VL序列,描述于美国专利8,153,765中。Isatuximab的VH和VL可表达为IgG1/κ。

[0102] SEQ ID NO 21:

[0103] QVQLVQSGAEVAKPGTSVKLSCKASGYTFTDYWMQWVKQRPGQGLEWIGT

[0104] IYPGDGDTGYAQKFQGKATLTADKSSKTVYMHLSSLASEDSAVYYCARGD

[0105] YYGSNSLDYWGQGTSVTVSS

[0106] SEQ ID NO:22:

[0107]

DIVMTQSHLSMSTSLGDPVSITCKASQDVSTVVAWYQQKPGQSPRRLIYSASYRYIGVPDRFTGSGAGTDFTFTISSVQAEDLAVYYCQQHYSPPYTFGGGTKLEIK。

[0108] 可用于本发明方法的其它示例性抗CD38抗体包括描述于国际专利公布WO05/103083、国际专利公布WO06/125640、国际专利公布WO07/042309、国际专利公布WO08/047242或国际专利公布WO14/178820中的那些。

[0109] 本文所述的本发明的另一个实施方案,并且其处在下文所列所有带编号的实施方案的一些实施方案中,是治疗患有急性髓系白血病(AML)的受治疗者的方法,该方法包括向对其有需要的受治疗者施用抗CD38抗体足以治疗AML的一段时间,该抗CD38抗体包含分别为SEQ ID NO:15和16的重链可变区(VH)和轻链可变区(VL)。

[0110] 本文所述的本发明的另一个实施方案,并且其处在下文所列所有带编号的实施方案的一些实施方案中,是治疗患有急性髓系白血病(AML)的受治疗者的方法,该方法包括向对其有需要的受治疗者施用抗CD38抗体足以治疗AML的一段时间,该抗CD38抗体包含分别为SEQ ID NO:17和18的重链可变区(VH)和轻链可变区(VL)。

[0111] 本文所述的本发明的另一个实施方案,并且其处在下文所列所有带编号的实施方案的一些实施方案中,是治疗患有急性髓系白血病(AML)的受治疗者的方法,该方法包括向对其有需要的受治疗者施用抗CD38抗体足以治疗AML的一段时间,该抗CD38抗体包含分别为SEQ ID NO:19和20的重链可变区(VH)和轻链可变区(VL)。

[0112] 本文所述的本发明的另一个实施方案,并且其处在下文所列所有带编号的实施方案的一些实施方案中,是治疗患有急性髓系白血病(AML)的受治疗者的方法,该方法包括向对其有需要的受治疗者施用抗CD38抗体足以治疗AML的一段时间,该抗CD38抗体包含分别为SEQ ID NO:21和22的重链可变区(VH)和轻链可变区(VL)。

[0113] 抗体的Fc部分可介导抗体效应子功能,诸如抗体依赖性细胞介导的细胞毒性(ADCC)、抗体依赖性细胞吞噬作用(ADCP)或补体依赖性细胞毒性(CDC)。这种功能可通过如下方式介导:Fc效应子域结合于具有吞噬或裂解活性的免疫细胞上的Fc受体,或Fc效应子域结合于补体系统的成分。通常,Fc结合细胞或补体成分所介导的效应导致靶细胞(例如,表达CD38的细胞)的抑制和/或耗竭。人IgG同种型IgG1、IgG2、IgG3和IgG4表现出效应子功能的差异能力。ADCC可由IgG1和IgG3介导,ADCP可由IgG1、IgG2、IgG3和IgG4介导,并且CDC可由IgG1和IgG3介导。

[0114] 在本文所述的方法中以及在下文所列所有带编号的实施方案的一些实施方案中,抗CD38抗体具有IgG1、IgG2、IgG3或IgG4同种型。

[0115] 在本文所述的方法中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体通过细胞凋亡来诱导杀伤表达CD38的AML细胞。

[0116] 用于本文所述方法中以及下文所列所有带编号的实施方案的一些实施方案中的抗CD38抗体，可通过细胞凋亡来诱导杀伤AML细胞。用于评估细胞凋亡的方法是熟知的，并且包括例如使用标准方法的膜联蛋白IV染色。用于本发明的方法中的抗CD38抗体可在约10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％、95％或100％的细胞中诱导细胞凋亡。

[0117] 在本文所述的方法中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38通过ADCC来诱导杀伤表达CD38的AML细胞。

[0118] 在本文所述的方法中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38通过CDC来诱导杀伤表达CD38的AML细胞。

[0119] 在本文所述的方法中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体通过ADCP来诱导杀伤表达CD38的AML细胞。

[0120] 在本文所述的方法中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体通过ADCC和CDC来诱导杀伤表达CD38的AML细胞。

[0121] “抗体依赖性细胞毒性”、“抗体依赖性细胞介导的细胞毒性”或“ADCC”是诱导细胞死亡的机制，该机制依赖于抗体包被靶细胞与具有裂解活性的效应细胞(诸如自然杀伤细胞、单核细胞、巨噬细胞和中性粒细胞)经由效应细胞上表达的Fcγ受体(FcγR)发生的相互作用。例如，NK细胞表达FcγRIIIa，而单核细胞表达FcγRI、FcγRII和FcvRIIIa。效应细胞通过分泌膜孔形成蛋白和蛋白酶而具有的活性会导致抗体包被靶细胞诸如表达CD38的细胞发生死亡。为评估抗CD38抗体的ADCC活性，可将抗体与免疫效应细胞联合加入到表达CD38的细胞中，所述免疫效应细胞可被抗原抗体复合物激活，从而使靶细胞发生细胞溶解。通常根据从裂解的细胞中释放的标记物(例如放射性底物、荧光染料或天然细胞内蛋白质)来检测细胞溶解。用于此类测定法的示例性效应细胞包括外周血单核细胞(PBMC)和NK细胞。示例性靶细胞包括表达CD38的Daudi细胞(ATCC® CCL-213™)或B细胞白血病或淋巴瘤肿瘤细胞。在示例性测定法中，用20μCi的^{51}Cr标记靶细胞2小时，然后充分冲洗。可将靶细胞的细胞浓度调节到1×10^{6}个细胞/ml，并添加各种浓度的抗CD38抗体。以40:1的效应细胞:靶细胞比率添加Daudi细胞，由此开始测定。在37℃下温育3小时后，通过离心终止测定，然后在闪烁计数器中测量从裂解的细胞中释放的^{51}Cr。可按通过将3％高氯酸加入靶细胞中诱导的最大裂解％来计算细胞毒性的百分比。用于本发明的方法中的抗CD38抗体可诱导ADCC达对照(通过3％高氯酸诱导的细胞裂解)的约20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％、95％或100％。

[0122] “抗体依赖性细胞吞噬作用”(“ADCP”)是指通过吞噬细胞(诸如巨噬细胞或树突细胞)的内化作用消除抗体包被靶细胞的机制。可通过如下方式评估ADCP:使用单核细胞来源的巨噬细胞作为效应细胞，并使用表达CD38的Daudi细胞(ATCC® CCL-213™)或者B细胞白血病或淋巴瘤肿瘤细胞作为靶细胞，所述靶细胞经工程化以表达GFP或其它标记分子。效应细胞:靶细胞比率可为例如4:1。可在含或不含抗CD38抗体的情况下，将效应细胞与靶细胞一起温育4小时。在温育后，可使用accutase分离细胞。可使用偶联至荧光标记物的抗CD11b抗体和抗CD14抗体鉴定巨噬细胞，并且可使用标准方法基于$CD11^{+}CD14^{+}$巨噬细胞中的

GFP荧光％确定吞噬百分比。用于本发明的方法中的抗CD38抗体可诱导ADCP达约20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％、95％或100％。

[0123] “补体依赖性细胞毒性”或“CDC”是指诱导细胞死亡的机制，其中靶结合抗体的Fc效应子域结合并激活补体成分C1q，继而激活补体级联，导致靶细胞死亡。补体的激活也可导致补体成分沉积在靶细胞表面上，这些补体成分通过结合白细胞上的补体受体(例如，CR3)来促进ADCC。可例如通过如下方式测量表达CD38的细胞的CDC：将Daudi细胞以1×10^5个细胞/孔(50μl/孔)接种到RPMI-B(补充有1％BSA的RPMI)中，将50μl抗CD38抗体以0-100μg/ml之间的最终浓度加入到孔中，将反应在室温下温育15分钟，将11μl的混合人血清加入到孔中，然后将反应在37℃下温育45分钟。可使用标准方法在FACS测定法中检测碘化丙啶染色细胞％作为裂解细胞百分比(％)。用于本发明的方法中的抗CD38抗体可诱导CDC达约20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％、95％或100％。

[0124] 单克隆抗体诱导ADCC的能力可通过工程化其寡糖成分来增强。人IgG1或IgG3在Asn297处被熟知的双分枝G0、G0F、G1、G1F、G2或G2F形式的大多数聚糖N-糖基化。由未经工程化的CHO细胞产生的抗体通常具有约至少85％的聚糖岩藻糖含量。从连接到Fc区的双分枝复合物型低聚糖去除核心岩藻糖，可经由改善的FcγRIIIa结合来增强抗体的ADCC，而不会改变抗原结合或CDC活性。此类mAb可使用据报道能引起具有双分枝复合物型Fc低聚糖的相对较高去岩藻糖基化抗体的成功表达的不同方法实现，诸如：控制培养物渗透压(Konno等人，Cytotechnology 64:249-65,2012)，应用变体CHO细胞系Lec13作为宿主细胞系(Shields等人，J Biol Chem 277:26733-26740,2002)，应用变体CHO细胞系EB66作为宿主细胞系(Olivier等人，MAbs;2(4),2010;印刷前电子版;PMID:20562582)，应用大鼠杂交瘤细胞系YB2/0作为宿主细胞系(Shinkawa等人，J Biol Chem 278:3466-3473,2003)，引入特异性针对α1,6-岩藻糖基转移酶(FUT8)基因的小干扰RNA(Mori等人，Biotechnol Bioeng88:901-908,2004)，或共表达β-1,4-N-乙酰氨基葡萄糖转移酶III和高尔基体α-甘露糖苷酶II或强效α-甘露糖苷酶I抑制剂几夫碱(kifunensine)(Ferrara等人，J Biol Chem281:5032-5036,2006，Ferrara等人，Biotechnol Bioeng 93:851-861,2006;Xhou等人，Biotechnol Bioeng99:652-65,2008)。由用于本发明的方法中以及下文所列所有带编号的实施方案的一些实施方案中的抗CD38抗体所引发的ADCC，也可通过抗体Fc中的某些置换来增强。示例性置换为例如在氨基酸位置256、290、298、312、356、330、333、334、360、378或430(根据EU索引对残基进行编号)处的置换，如美国专利6,737,056中所述。

[0125] 在本文所述的一些方法中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体包含抗体Fc中的置换。

[0126] 在本文所述的一些方法中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体包含抗体Fc中的氨基酸位置256、290、298、312、356、330、333、334、360、378或430(根据EU索引对残基进行编号)处的置换。

[0127] 在本文所述的一些方法中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体具有岩藻糖含量大约在0％至约15％之间(例如15％、14％、13％、12％、11％、10％、9％、8％、7％、6％、5％、4％、3％、2％、1％或0％)的双分枝聚糖结构。

[0128] 在本文所述的一些方法中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体具有岩藻糖含量为约50％、40％、45％、40％、35％、30％、25％、20％、15％、14％、13％、12％、11％、10％、9％、8％、7％、6％、5％、4％、3％、2％、1％或0％的双分枝聚糖结构。

[0129] Fc中的置换和减少的岩藻糖含量可增强抗CD38抗体的ADCC活性。

[0130] “岩藻糖含量”意指Asn297处糖链内的岩藻糖单糖的量。岩藻糖的相对量为含岩藻糖的结构相对于所有糖结构的百分比。这些结构可通过多种方法进行表征和定量，例如：1)使用对经N-糖苷酶F处理的样本(例如，复合结构、混合结构及低聚甘露糖结构和高甘露糖结构)的MALDI-TOF，如国际专利公布WO2008/077546中所述；2)酶促释放Asn297聚糖，随后衍生化并通过采用荧光检测的HPLC(UPLC)和/或HPLC-MS(UPLC-MS)进行检测/定量；3)对天然或经还原的mAb进行完整蛋白分析，同时进行或不进行用Endo S或其它酶对Asn297聚糖的处理，所述酶在第一GlcNAc单糖和第二GlcNAc单糖之间引发裂解，留下连接于第一GlcNAc的岩藻糖；4)通过酶消化(例如，胰蛋白酶或内肽酶Lys-C)将mAb消化为成分肽，随后通过HPLC-MS(UPLC-MS)进行分离、检测和定量；或5)用PNGase F在Asn 297处进行特异性酶促去糖基化，从而将mAb低聚糖与mAb蛋白分离。可通过各种互补技术在所释放的低聚糖上标记荧光团，分离并鉴定所释放的低聚糖，这些技术允许：采用基质辅助激光解吸电离(MALDI)质谱法，通过比较实验质量与理论质量来对聚糖结构进行精细表征；通过离子交换HPLC(GlycoSep C)确定唾液酸化程度；通过正相HPLC(GlycoSep N)，根据亲水性标准分离和定量低聚糖形式；以及通过高效毛细管电泳激光诱导荧光(HPCE-LIF)分离和定量低聚糖。

[0131] 如本申请中所用，“低岩藻糖”或“低岩藻糖含量”是指抗体的岩藻糖含量为约0％-15％。

[0132] 如本文所用，“正常岩藻糖”或“正常岩藻糖含量”是指抗体的岩藻糖含量大约高于50％，通常大约高于60％、70％、80％或高于85％。

[0133] 用于本文所述方法中以及下文所列所有带编号的实施方案的一些实施方案中的抗CD38抗体，可通过CD38酶活性调节来诱导杀伤AML细胞。CD38是多功能胞外酶，其具有ADP核糖基环化酶活性，催化由NAD形成环ADP核糖(cADPR)和ADPR的过程。CD38还催化酸性条件下$NADP^+$的烟酰胺基团与烟酸的交换，产生$NAADP^+$(烟酸-腺嘌呤二核苷酸磷酸)。用于本发明方法中的抗CD38抗体对人CD38的酶活性的调节，可在Graeff等人，J.Biol.Chem.269，30260-30267(1994)所述的测定法中进行测量。例如，可将底物NGD^+与CD38一起温育，并且可在添加各种浓度的抗体后的不同时间点，通过分光光度法，在340nM的激发波长和410nM的发射波长下监测环GDP核糖(cGDPR)生成的调节。可根据Munshi等人，J.Biol.Chem.275，21566-21571(2000)所述的HPLC方法确定cADPR合成的抑制。用于本文所述的本发明的方法中以及下文所列所有带编号的实施方案的一些实施方案中的抗CD38抗体可抑制CD38酶活性达至少约20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％、95％或100％。

[0134] 在本文所述的本发明的一些方法中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体包含分别为SEQ ID NO:6、7和8的重链互补决定区(HCDR)1(HCDR1)、2(HCDR2)和3(HCDR3)序列。

[0135] 在本文所述的本发明的一些方法中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体包含分别为SEQ ID NO:9、10和11的轻链互补决定区(LCDR)1(LCDR1)、2(LCDR2)和3(LCDR3)序列。

[0136] 在本文所述的本发明的一些方法中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体包含分别为SEQ ID NO:6、7和8的重链互补决定区(HCDR)1(HCDR1)、2(HCDR2)和3(HCDR3)序列，以及分别为SEQ ID NO:9、10和11的轻链互补决定区(LCDR)1(LCDR1)、2(LCDR2)和3(LCDR3)序列。

[0137] 在本文所述的本发明的一些方法中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体包含SEQ ID NO:4的重链可变区(VH)和SEQ ID NO:5的轻链可变区(VL)。

[0138] 在本文所述的本发明的一些方法中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体包含SEQ ID NO:12的重链和SEQ ID NO:13的轻链。

[0139] 在本文所述的本发明的一些方法中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体包含氨基酸序列与SEQ ID NO:12的氨基酸序列具有95％、96％、97％、98％或99％同一性的重链以及氨基酸序列与SEQ ID NO:13的氨基酸序列具有95％、96％、97％、98％或99％同一性的轻链。

[0140] 与包含SEQ ID NO:12的重链和SEQ ID NO:13的轻链的抗体基本上相同的抗体可用于本发明的方法中。如本文所用，“基本上相同”意指被比较的两个抗体重链或轻链氨基酸序列是相同的或具有“非显著性差异”。非显著性差异是指抗体重链或轻链中有1、2、3、4、5、6、7、8、9、10、11、12、13、14或15个氨基酸被置换，这些置换不会对抗体的特性造成不利影响。百分比同一性可以例如通过使用Vector NTI v.9.0.0(Invitrogen,Carlsbad,CA)的AlignX模块的默认设置的配对比对而确定。本发明的蛋白质序列可以用作查询序列以针对公共或专利数据库进行检索，以例如鉴定相关序列。用来进行此类检索的示例性程序是使用默认设置的XBLAST或BLASTP程序(http_//www_ncbi_nlm/nih_gov)或GenomeQuest™(GenomeQuest,Westborough,MA)软件包。可对用于本发明方法中的抗CD38抗体进行的示例性置换为例如用具有相似电荷、疏水性或立体化学特性的氨基酸进行的保守置换。还可作出保守置换以改善抗体特性，例如稳定性或亲和力，或改善抗体效应子功能。可例如对抗CD38抗体的重链或轻链作出1、2、3、4、5、6、7、8、9、10、11、12、13、14或15个氨基酸置换。此外，重链或轻链中的任何天然残基还可以用丙氨酸来置换，如此前对于丙氨酸扫描诱变所述的(MacLennan等人，Acta Physiol Scand Suppl 643:55-67,1998;Sasaki等人，Adv Biophys 35:1-24,1998)。所需的氨基酸置换可以由本领域技术人员在需要此类置换时确定。氨基酸置换可以例如通过PCR诱变来进行(美国专利4,683,195)。可使用熟知方法生成变体文库，例如使用随机(NNK)或非随机密码子(例如DVK密码子，其编码11个氨基酸(Ala、Cys、Asp、Glu、Gly、Lys、Asn、Arg、Ser、Tyr、Trp)，并在文库中筛选具有所需特性的变体。可使用本文所述的方法检测所生成的变体对CD38的结合，及其诱导细胞凋亡的能力，或调节CD38酶活性的能力。

[0141] 在本文所述的方法中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体可以一系列亲和力(K_D)结合人CD38。在根据本发明的一个实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体结合CD38的K_D等于或小于约

1×10^{-8}M,例如5×10^{-9}M、1×10^{-9}M、5×10^{-10}M、1×10^{-10}M、5×10^{-11}M、1×10^{-11}M、5×10^{-12}M、1×10^{-12}M、5×10^{-13}M、1×10^{-13}M、5×10^{-14}M、1×10^{-14}M或5×10^{-15}M、或者其中的任何范围或值,这由本领域技术人员实施的表面等离子共振或Kinexa方法确定。一种示例性亲和力等于或小于1×10^{-8}M。另一种示例性亲和力等于或小于1×10^{-9}M。

[0142] 在一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中,抗CD38抗体为双特异性抗体。可将现有抗CD38抗体的VL和/或VH区或者如本文所述从头鉴定的VL和VH区工程化成双特异性全长抗体。可使用诸如以下专利中所述的那些技术,通过调节抗体Fc中的CH3相互作用以形成双特异性抗体,从而制备此类双特异性抗体:美国专利7,695,936;国际专利公布WO04/111233;美国专利公布US2010/0015133;美国专利公布US2007/0287170;国际专利公布WO2008/119353;美国专利公布US2009/0182127;美国专利公布US2010/0286374;美国专利公布US2011/0123532;国际专利公布WO2011/131746;国际专利公布WO2011/143545;或美国专利公布US2012/0149876。

[0143] 例如,根据国际专利公布WO2011/131746所述的方法,本发明的双特异性抗体可在无细胞环境中,通过在两个单特异性同源二聚抗体的CH3区域中导入非对称突变并由两个亲本单特异性同源二聚抗体在还原条件下形成双特异性异源二聚抗体而体外生成,该还原条件允许二硫键异构化。在该方法中,第一单特异性二价抗体(例如抗CD38抗体)和第二单特异性二价抗体经工程化以在CH3域具有某些置换,这提高异源二聚体的稳定性;抗体在还原条件下一起孵育,这足以允许铰链区的半胱氨酸发生二硫键异构化;从而通过Fab臂交换生成双特异性抗体。孵育条件最理想地可恢复到非还原条件。可使用的示例性还原剂为2-巯基乙胺(2-MEA)、二硫苏糖醇(DTT)、二硫赤藓糖醇(DTE)、谷胱甘肽、三(2-羧乙基)膦(TCEP)、L-半胱氨酸和β-巯基乙醇,优选地选自:2-巯基乙胺、二硫苏糖醇和三(2-羧乙基)膦。例如,在至少25mM 2-MEA的存在下或至少0.5mM二硫苏糖醇的存在下,在5-8的pH例如pH7.0或pH7.4以及至少20℃的温度下,可孵育至少90分钟。

[0144] 可用于双特异性抗体的第一重链和第二重链的示例性CH3突变是K409R和/或F405L。

[0145] 可在其中结合本发明的抗体的VL区和/或VH区的另外的双特异性结构,是例如双可变域免疫球蛋白(Dual Variable Domain Immunoglobulins,DVD)(国际专利公布WO2009/134776),或包括多种二聚化域以连接具有不同特异性的两个抗体臂的结构,诸如亮氨酸拉链或胶原二聚化域(国际专利公布WO2012/022811、美国专利5,932,448、美国专利6,833,441)。DVD是全长抗体,包含具有VH1-接头-VH2-CH结构的重链和具有VL1-接头-VL2-CL结构的轻链;接头是任选的。

[0146] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中,抗CD38抗体缀合至毒素。缀合方法和合适的毒素是熟知的。

[0147] AML诊断由医生根据可用的指南如根据世界卫生组织(World Health Organization,WHO)的AML分类(Brunning等人,World Health Organization Classificaiton of Tumors,3,第77-80页;Jaffe等人编辑,Pathology and Genetics ofTumours ofHaematopoietic and Lymphoid Tissues)以及根据可用的指南如National Comprehensive Cancer Network(http://_www_nccn.org/_professionals/_physician_gls/_f_guidelines_asp#site)网站上的指南进行。WHO分类包括临床特征、细胞遗传学、免

疫显型、形态学和遗传学，用于定义具有治疗和预后相关性的生物学上同源的亚组，并且将AML分成四个主要亚型：伴有重现性遗传异常的AML、伴有多系病变的AML、治疗相关性AML、以及分类不明的AML。

[0148] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中，AML是伴有至少一种遗传异常的AML。

[0149] AML可能与染色体8和21之间的易位、染色体16中的易位或倒位、染色体15和17之间的易位、或染色体11的改变相关。

[0150] 与AML相关的常见染色体重排为易位t(8;21)(q22;q22)(AML1/ETO)、inv(16)(p13;q22)或t(16;16)(p13;q22)；(CBFβ/MYH11)或t(15;17)(q22;q12)；(PML/RARA)。伴有这些有利的染色体易位的患者可较易于治疗并实现较高的完全缓解(CR)率。

[0151] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中，AML与染色体8和21之间的易位、染色体16中的易位或倒位、染色体15和17之间的易位、或染色体11的改变相关。

[0152] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中，AML与染色体异常t(8;21)(q22;q22)(AML1/ETO)、inv(16)(p13;q22)或t(16;16)(p13;q22)；(CBFβ/MYH11)或t(15;17)(q22;q12)；(PML/RARA)相关。

[0153] 已经识别出在多个基因中的体细胞突变与AML发病机制相关。这些包括在fms-相关的酪氨酸激酶3(FLT3)、核仁磷酸蛋白(NPM1)、异柠檬酸脱氢酶1(IDH1)、异柠檬酸脱氢酶2(IDH2)、DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)、CCAAT/增强子结合蛋白α(CEBPA)、U2小核RNA辅助因子1(U2AF1)、zeste 2多梳抑制复合物2亚基的增强子(EZH2)、染色体1A的结构维持(SMC1A)和染色体3的结构维持(SMC3)(The Cancer Genome Atlas Research Network;N Engl J Med 368:2059-74,2013)中的突变。

[0154] 在FLT3基因中的激活突变已经在大约20-30％的新诊断AML患者中得到描述。这些包括FLT3-ITD内部串联重复突变，由复制和串联插入FLT3基因的近膜域部分(Schnittger等人，Blood 100:59-66,2002)引起，以及在FLT3激酶域中的D835突变。患有FLT3-ITD突变的患者看起来具有降低的总体生存(OS)率以及提高的复发率(Kottaridis等人，Blood 98:1752-9,2001;Yanada等人，Leukemia 19:1345-9,2005)。

[0155] IDH1和IDH2中的突变存在于约15％的新诊断患者中。IDH1突变包括置换R132H、R132X(X为任意氨基酸)并且R100Q/R104V/F108L/R119Q/I130V和IDH2突变包括置换R140Q和R172。IDH1/2突变与预后不良相关，不同的是$IDH2^{R140Q}$一定程度上与延长生存相关(Molenaar等人，Biochim Biophys Acta 1846:326-41,2014)。IDH1/2突变频率随着疾病进展而提高(Molenaar等人，Biochim Biophys Acta 1846:326-41,2014)。

[0156] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中，AML与在fms-相关的酪氨酸激酶3(FLT3)、核仁磷酸蛋白(NPM1)、异柠檬酸脱氢酶1(IDH1)、异柠檬酸脱氢酶2(IDH2)、DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)、CCAAT/增强子结合蛋白α(CEBPA)、U2小核RNA辅助因子1(U2AF1)、zeste 2多梳抑制复合物2亚基的增强子(EZH2)、染色体1A的结构维持(SMC1A)和染色体3的结构维持(SMC3)中的一个或多个突变相关。

[0157] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实

施方案中,AML与在fms-相关的酪氨酸激酶3(FLT3)中的一个或多个突变相关。

[0158] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中,AML与FLT3-ITD相关。

[0159] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中,AML与在异柠檬酸脱氢酶1(IDH1)或异柠檬酸脱氢酶2(IDH2)中的一个或多个突变相关。

[0160] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中,AML与在异柠檬酸脱氢酶1(IDH1)中的突变R132H、R132X或R100Q/R104V/F108L/R119Q/I130V相关。

[0161] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中,AML与在异柠檬酸脱氢酶2(IDH2)中的突变R140Q和R172相关。

[0162] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中,AML是伴有多系病变的AML。

[0163] 与多系病变相关的AML特征在于在两个或更多个髓系细胞谱系中的病变、以及在血液或骨髓中增加至少20%的母细胞。

[0164] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中,AML是治疗相关性AML。

[0165] 治疗相关性AML是之前的化学疗法和/或放射治疗的结果,并且可在暴露于诱变剂若干年后发生。超过90%的患有治疗相关性AML的患者表现出染色体异常,包括染色体5和/或7的那些。

[0166] 染色体重排可使用熟知的方法例如荧光原位杂交、染色体核型分析、Southern印迹或测序进行鉴定。

[0167] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中,AML为未分化型AML(M0)、微成熟型AML(M1)、成熟型AML(M2)、急性髓单核细胞白血病(M4)、急性单核细胞白血病(M5)、急性红白血病(M6)、急性巨核细胞白血病(M7)、急性嗜碱性白血病、急性全髓纤维化或髓系肉瘤。

[0168] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中,AML处于缓解中。

[0169] 处于缓解中的AML通常定义为正常细胞的骨髓,具有少于5%的母细胞,正常外周血计数>100,000/mm^3个血小板和>1,000/mm^3个中性粒细胞。

[0170] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中,AML为复发的或难治的。

[0171] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中,患有AML的患者已经历伊达比星、阿糖胞苷或羟基脲治疗。

[0172] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中,AML是成人AML。

[0173] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中,AML是小儿AML。

[0174] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实

施方案中，抗CD38抗体作为缓解诱导、缓解后或维持疗法来施用。

[0175] 可使用各种定性和/或定量方法确定已经复发的受治疗者是否对采用药物或疗法进行的治疗表现出抵抗性，或者已发展出对这种治疗的抵抗性，或易发展出对这种治疗的抵抗性。可能与复发和/或抵抗性相关的症状包括例如：患者健康状况的下降或平稳，肿瘤尺寸或肿瘤负荷的增加，癌细胞数量的增多，肿瘤或肿瘤细胞的生长下降受阻或减缓，和/或身体中的癌细胞从一个部位扩散到其它器官、组织或细胞。与肿瘤相关的各种症状的复发或恶化也可指示受治疗者已复发或已发展出或易发展出对药物或疗法的抵抗性。与癌相关的症状可根据癌的类型而有所变化。例如，与AML相关的症状可包括虚弱、疲劳、头晕感或冷感、头疼、频繁流鼻血、过多的瘀紫或牙龈出血。

[0176] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体与至少一种另外的疗法联合施用。

[0177] AML可用阿糖胞苷(胞嘧啶阿拉伯糖苷酶、或ara-C)和/或蒽环类抗生素药物诸如多柔比星、佐柔比星、道诺霉素、伊达比星和米托蒽醌治疗。可用于治疗AML的其它化疗药物包括羟基脲(Hydrea®)、地西他滨(Dacogen®)、克拉屈滨(Leustatin®,2-CdA)、氟达拉滨(Fludara®)、拓朴替康、依托泊苷(VP-16)、6-硫鸟嘌呤(6-TG)、皮质类固醇药如泼尼松或地塞米松(Decadron®)、甲氨蝶呤(MTX)、6-巯嘌呤(6-MP)或阿扎胞苷(Vidaza®)。

[0178] 可用于治疗AML的其它药物为全反式视黄酸(ATRA)、维A酸、或Vesanoid®以及三氧化二砷(ATO，Trisenox®)。ATRA和三氧化二砷可用于治疗急性早幼粒细胞白血病。

[0179] 在一些实施方案中，抗CD38抗体与阿糖胞苷、佐柔比星/道诺霉素、伊达比星、米托蒽醌、羟基脲、地西他滨、克拉屈滨、氟达拉滨、拓朴替康、依托泊苷6-硫鸟嘌呤、皮质类固醇、泼尼松、地塞米松、甲氨蝶呤、6-巯嘌呤或阿扎胞苷联合施用于患者。

[0180] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体与地西他滨联合施用于患者。

[0181] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体与阿糖胞苷和多柔比星联合施用于患者。

[0182] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中，受治疗者已接受或将接受放射疗法。

[0183] 放射疗法可为外照射、调强放射疗法(IMRT)、聚焦放射、或任何形式的放射外科手术，包括伽玛刀、射波刀、直线加速器和组织间放射(例如，植入放射性粒子、GliaSite球囊)、和/或与外科手术联合。

[0184] 可使用的聚焦放射方法包括立体定向放射外科手术、分次立体定向放射外科手术、和调强放射疗法(IMRT)。明显的是立体定向放射外科手术涉及将放射精确递送至肿瘤组织如脑瘤，同时避开周围的非肿瘤正常组织。使用立体定向放射外科手术施用的放射剂量可变化，通常从1Gy至约30Gy，并且可包括中间范围，包括例如1至5、10、15、20、25、最多30Gy的剂量。因为有无创固定装置，立体定向放射不需要在单次治疗中递送。治疗计划可每天可靠地重复，从而允许递送多个分次放射剂量。当用于经一段时间治疗肿瘤时，放射外科手术称为“分次立体定向放射外科手术”或FSR。相比之下，立体定向放射外科手术是指一次性治疗。分次立体定向放射外科手术可导致高治疗比，即，高比率的肿瘤细胞杀灭和对正常

组织的低影响。肿瘤和正常组织对单次高放射剂量和多次较小放射剂量的反应不同。与若干次较小放射剂量相比，单次大放射剂量可杀伤更多正常组织。因此，多次较小放射剂量能够杀伤更多肿瘤细胞，同时避开正常组织。使用分次立体定向放射施用的放射剂量可变化，从1Gy至约50Gy，并且可包括中间范围，包括例如1至5、10、15、20、25、30、40、最多50Gy的少分次剂量。也可使用调强放射疗法(IMRT)。IMRT是一种高精度三维适形放射治疗(3DCRT)的高级模式，其使用计算机控制的直线加速器以递送精确的放射剂量至恶性肿瘤或肿瘤内的特定区域。在3DCRT中，每个放射束的剖面使用多叶准直器(MLC)适形于射野方向观视(BEV)的目标外形，从而产生多个射束。IMRT允许通过调节在多个小区中的放射束强度，使放射剂量较精确地适配于肿瘤的三维(3-D)形状。因此，IMRT允许较高的放射剂量集中在肿瘤内的区域，同时最小化周围的正常关键结构的剂量。IMRT改善适配治疗区与凹肿瘤形状的能力，例如当肿瘤围绕易受伤害的结构时，诸如脊髓或主要器官或血管。

[0185] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中，受治疗者正经受造血干细胞移植(HSCT)。

[0186] 在本文所述的本发明一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中，HSCT是异源的、自体同源的或同基因的，即，供体为孪生者。自体同源的HSCT包括从受治疗者提取HSC并冷冻获得的HSC。在清髓后，将受治疗者的存储HSC移植到受治疗者。异源HSCT涉及从匹配受治疗者的HLA型的异源HSC供体中获取HSC。

[0187] “造血干细胞移植”是来源于骨髓(在这种情况下称为骨髓移植)、血液(例如外周血和脐带血)、或羊水的血液干细胞的移植。

[0188] “经受造血干细胞移植”是指患者过去已经接受、正在接受、或将要接受HSCT。

[0189] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中，患者在HSCT之前已经完成化学治疗和/或放射治疗。

[0190] 患者可在HSCT之前进行化学治疗和/或放射治疗(所谓的移植前准备)以在移植前清除患者的一些或所有造血细胞。在异源HSCT的情况下，患者可能还要经历免疫抑制剂治疗。一种示例性的移植前准备疗法是高剂量的美法仑(参见例如Skinner等人，Ann Intern Med 140:85-93，2004；Gertz等人，Bone Marrow Transplant 34:1025-31，2004；Perfetti等人，Haematologica 91:1635-43，2006)。可在移植前治疗中使用的放射治疗可根据本领域中通常已知的方案进行。也可同时、依序或分开提供放射治疗与抗CD38抗体。

[0191] 在本文所述的一些实施方案中以及在下文所列所有带编号的实施方案的一些实施方案中，患有AML的受治疗者对于CD16的位置158处的苯丙氨酸是纯合的(Fcγ RIIIa-158F/F基因型)，或对于CD16的位置158处的缬氨酸和苯丙氨酸是杂合的(Fcγ RIIIa-158F/V基因型)。CD16也称为Fcγ受体IIIa(Fcγ RIIIa)或低亲和力免疫球蛋白γ Fc区受体III-A同种型。已证实Fcγ RIIIa蛋白残基位置158处的缬氨酸/苯丙氨酸(V/F)多态性会影响Fcγ RIIIa对人IgG的亲和力。与Fcγ RIIIa-158V/V相比，具有Fcγ RIIIa-158F/F或Fcγ RIIIa-158F/V多态性的受体展示出减弱的Fc接合及因此减轻的ADCC。人N-连接的寡糖上不含或含少量岩藻糖，提高了抗体诱导ADCC的能力，这是由于抗体与人Fcγ RIIIa(CD16)的结合得以改善(Shields等人，J Biol Chem 277:26733-40，2002)。可使用常规方法分析患者的Fcγ RIIIa多态性。

[0192] 本发明还提供了用于治疗患有AML的受治疗者的方法，该方法包括向对其有需要

的患者施用与人CD38(SEQ ID NO:1)的区域SKRNIQFSCKNIYR(SEQ ID NO:2)和区域EKVQTLEAWVIHGG(SEQ ID NO:3)结合的抗CD38抗体,其中受治疗者在CD16的位置158处具有苯丙氨酸纯合或者在CD16的位置158处具有缬氨酸和苯丙氨酸杂合。

[0193] 本发明也提供用于治疗患有AML的受治疗者的抗CD38抗体,其中受治疗者具有在fms-相关的酪氨酸激酶3(FLT3)中的突变。

[0194] 本发明也提供用于治疗患有AML的受治疗者的抗CD38抗体,其中受治疗者具有FLT3-ITD突变。

[0195] 本发明也提供用于治疗患有AML的受治疗者的抗CD38抗体,其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的突变。

[0196] 本发明也提供用于治疗患有AML的受治疗者的抗CD38抗体,其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的R140Q突变。

[0197] 本发明也提供用于治疗患有AML的受治疗者的抗CD38抗体,其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的突变。

[0198] 本发明也提供用于治疗患有AML的受治疗者的抗CD38抗体,其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的R882H突变。

[0199] 本发明也提供用于与第二治疗剂联合治疗患有AML的受治疗者的抗CD38抗体,其中受治疗者具有在fms-相关的酪氨酸激酶3(FLT3)中的突变。

[0200] 本发明也提供用于与第二治疗剂联合治疗患有AML的受治疗者的抗CD38抗体,其中受治疗者具有FLT3-ITD突变。

[0201] 本发明也提供用于与第二治疗剂联合治疗患有AML的受治疗者的抗CD38抗体,其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的突变。

[0202] 本发明也提供用于与第二治疗剂联合治疗患有AML的受治疗者的抗CD38抗体,其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的R140Q突变。

[0203] 本发明也提供用于与第二治疗剂联合治疗患有AML的受治疗者的抗CD38抗体,其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的突变。

[0204] 本发明也提供用于与第二治疗剂联合治疗患有AML的受治疗者的抗CD38抗体,其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的R882H突变。

[0205] 本发明也提供用于与达克金联合治疗患有AML的受治疗者的抗CD38抗体,其中受治疗者具有在fms-相关的酪氨酸激酶3(FLT3)中的突变。

[0206] 本发明也提供用于与达克金联合治疗患有AML的受治疗者的抗CD38抗体,其中受治疗者具有FLT3-ITD突变。

[0207] 本发明也提供用于与达克金联合治疗患有AML的受治疗者的抗CD38抗体,其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的突变。

[0208] 本发明也提供用于与达克金联合治疗患有AML的受治疗者的抗CD38抗体,其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的R140Q突变。

[0209] 本发明也提供用于与达克金联合治疗患有AML的受治疗者的抗CD38抗体,其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的突变。

[0210] 本发明也提供用于与达克金联合治疗患有AML的受治疗者的抗CD38抗体,其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的R882H突变。

[0211] 本发明也提供用于与阿糖胞苷联合治疗患有AML的受治疗者的抗CD38抗体，其中受治疗者具有在fms-相关的酪氨酸激酶3(FLT3)中的突变。

[0212] 本发明也提供用于与阿糖胞苷联合治疗患有AML的受治疗者的抗CD38抗体，其中受治疗者具有FLT3-ITD突变。

[0213] 本发明也提供用于与阿糖胞苷联合治疗患有AML的受治疗者的抗CD38抗体，其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的突变。

[0214] 本发明也提供用于与阿糖胞苷联合治疗患有AML的受治疗者的抗CD38抗体，其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的R140Q突变。

[0215] 本发明也提供用于与阿糖胞苷联合治疗患有AML的受治疗者的抗CD38抗体，其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的突变。

[0216] 本发明也提供用于与阿糖胞苷联合治疗患有AML的受治疗者的抗CD38抗体，其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的R882H突变。

[0217] 本发明也提供用于与多柔比星联合治疗患有AML的受治疗者的抗CD38抗体，其中受治疗者具有在fms-相关的酪氨酸激酶3(FLT3)中的突变。

[0218] 本发明也提供用于与多柔比星联合治疗患有AML的受治疗者的抗CD38抗体，其中受治疗者具有FLT3-ITD突变。

[0219] 本发明也提供用于与多柔比星联合治疗患有AML的受治疗者的抗CD38抗体，其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的突变。

[0220] 本发明也提供用于与多柔比星联合治疗患有AML的受治疗者的抗CD38抗体，其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的R140Q突变。

[0221] 本发明也提供用于与多柔比星联合治疗患有AML的受治疗者的抗CD38抗体，其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的突变。

[0222] 本发明也提供用于与多柔比星联合治疗患有AML的受治疗者的抗CD38抗体，其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的R882H突变。

[0223] 本发明也提供用于与阿糖胞苷和多柔比星联合治疗患有AML的受治疗者的抗CD38抗体，其中受治疗者具有在fms-相关的酪氨酸激酶3(FLT3)中的突变。

[0224] 本发明也提供用于与阿糖胞苷和多柔比星联合治疗患有AML的受治疗者的抗CD38抗体，其中受治疗者具有FLT3-ITD突变。

[0225] 本发明也提供用于与阿糖胞苷和多柔比星联合治疗患有AML的受治疗者的抗CD38抗体，其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的突变。

[0226] 本发明也提供用于与阿糖胞苷和多柔比星联合治疗患有AML的受治疗者的抗CD38抗体，其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的R140Q突变。

[0227] 本发明也提供用于与阿糖胞苷和多柔比星联合治疗患有AML的受治疗者的抗CD38抗体，其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的突变。

[0228] 本发明也提供用于与阿糖胞苷和多柔比星联合治疗患有AML的受治疗者的抗CD38抗体，其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的R882H突变。

[0229] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于治疗患有AML的受治疗者，其中受治疗者具有在fms-相关的酪氨酸激酶3(FLT3)中的突变。

[0230] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于治

疗患有AML的受治疗者，其中受治疗者具有FLT3-ITD突变。

[0231] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于治疗患有AML的受治疗者，其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的突变。

[0232] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于治疗患有AML的受治疗者，其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的R140Q突变。

[0233] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于治疗患有AML的受治疗者，其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的突变。

[0234] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于治疗患有AML的受治疗者，其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的R882H突变。

[0235] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与第二治疗剂联合治疗患有AML的受治疗者，其中受治疗者具有在fms-相关的酪氨酸激酶3(FLT3)中的突变。

[0236] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与第二治疗剂联合治疗患有AML的受治疗者，其中受治疗者具有FLT3-ITD突变。

[0237] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与第二治疗剂联合治疗患有AML的受治疗者，其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的突变。

[0238] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与第二治疗剂联合治疗患有AML的受治疗者，其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的R140Q突变。

[0239] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与第二治疗剂联合治疗患有AML的受治疗者，其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的突变。

[0240] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与第二治疗剂联合治疗患有AML的受治疗者，其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的R882H突变。

[0241] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与达克金联合治疗患有AML的受治疗者，其中受治疗者具有在fms-相关的酪氨酸激酶3(FLT3)中的突变。

[0242] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与达克金联合治疗患有AML的受治疗者，其中受治疗者具有FLT3-ITD突变。

[0243] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与达克金联合治疗患有AML的受治疗者，其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的突变。

[0244] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与达克金联合治疗患有AML的受治疗者，其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的R140Q突变。

[0245] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与达克金联合治疗患有AML的受治疗者，其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的突变。

[0246] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与达克金联合治疗患有AML的受治疗者，其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的R882H突变。

[0247] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与阿糖胞苷联合治疗患有AML的受治疗者，其中受治疗者具有在fms-相关的酪氨酸激酶3(FLT3)中的突变。

[0248] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与阿糖胞苷联合治疗患有AML的受治疗者，其中受治疗者具有FLT3-ITD突变。

[0249] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与阿糖胞苷联合治疗患有AML的受治疗者，其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的突变。

[0250] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与阿糖胞苷联合治疗患有AML的受治疗者，其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的R140Q突变。

[0251] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与阿糖胞苷联合治疗患有AML的受治疗者，其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的突变。

[0252] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与阿糖胞苷联合治疗患有AML的受治疗者，其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的R882H突变。

[0253] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与多柔比星联合治疗患有AML的受治疗者，其中受治疗者具有在fms-相关的酪氨酸激酶3(FLT3)中的突变。

[0254] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与多柔比星联合治疗患有AML的受治疗者，其中受治疗者具有FLT3-ITD突变。

[0255] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与多柔比星联合治疗患有AML的受治疗者，其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的突变。

[0256] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与多柔比星联合治疗患有AML的受治疗者，其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的R140Q突变。

[0257] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与多柔比星联合治疗患有AML的受治疗者，其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的突变。

[0258] 本发明也提供抗CD38抗体，其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL，用于与多柔比星联合治疗患有AML的受治疗者，其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3

(DNMT3A)中的R882H突变。

[0259] 本发明也提供抗CD38抗体,其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL,用于与阿糖胞苷和多柔比星联合治疗患有AML的受治疗者,其中受治疗者具有在fms-相关的酪氨酸激酶3(FLT3)中的突变。

[0260] 本发明也提供抗CD38抗体,其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL,用于与阿糖胞苷和多柔比星联合治疗患有AML的受治疗者,其中受治疗者具有FLT3-ITD突变。

[0261] 本发明也提供抗CD38抗体,其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL,用于与阿糖胞苷和多柔比星联合治疗患有AML的受治疗者,其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的突变。

[0262] 本发明也提供抗CD38抗体,其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL,用于与阿糖胞苷和多柔比星联合治疗患有AML的受治疗者,其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的R140Q突变。

[0263] 本发明也提供抗CD38抗体,其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL,用于与阿糖胞苷和多柔比星联合治疗患有AML的受治疗者,其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的突变。

[0264] 本发明也提供抗CD38抗体,其包含SEQ ID NO:4的VH和SEQ ID NO:5的VL,用于与阿糖胞苷和多柔比星联合治疗患有AML的受治疗者,其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的R882H突变。

[0265] 本发明也提供抗CD38抗体,其包含SEQ ID NO:15的VH和SEQ ID NO:16的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在fms-相关的酪氨酸激酶3(FLT3)中的突变。

[0266] 本发明也提供抗CD38抗体,其包含SEQ ID NO:15的VH和SEQ ID NO:16的VL,用于治疗患有AML的受治疗者,其中受治疗者具有FLT3-ITD突变。

[0267] 本发明也提供抗CD38抗体,其包含SEQ ID NO:15的VH和SEQ ID NO:16的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的突变。

[0268] 本发明也提供抗CD38抗体,其包含SEQ ID NO:15的VH和SEQ ID NO:16的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的R140Q突变。

[0269] 本发明也提供抗CD38抗体,其包含SEQ ID NO:15的VH和SEQ ID NO:16的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的突变。

[0270] 本发明也提供抗CD38抗体,其包含SEQ ID NO:15的VH和SEQ ID NO:16的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的R882H突变。

[0271] 本发明也提供抗CD38抗体,其包含SEQ ID NO:17的VH和SEQ ID NO:18的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在fms-相关的酪氨酸激酶3(FLT3)中的突变。

[0272] 本发明也提供抗CD38抗体,其包含SEQ ID NO:17的VH和SEQ ID NO:18的VL,用于治疗患有AML的受治疗者,其中受治疗者具有FLT3-ITD突变。

[0273] 本发明也提供抗CD38抗体,其包含SEQ ID NO:17的VH和SEQ ID NO:18的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的突变。

[0274] 本发明也提供抗CD38抗体,其包含SEQ ID NO:17的VH和SEQ ID NO:18的VL,用于

治疗患有AML的受治疗者,其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的R140Q突变。

[0275] 本发明也提供抗CD38抗体,其包含SEQ ID NO:17的VH和SEQ ID NO:18的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的突变。

[0276] 本发明也提供抗CD38抗体,其包含SEQ ID NO:17的VH和SEQ ID NO:18的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的R882H突变。

[0277] 本发明也提供抗CD38抗体,其包含SEQ ID NO:19的VH和SEQ ID NO:20的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在fms-相关的酪氨酸激酶3(FLT3)中的突变。

[0278] 本发明也提供抗CD38抗体,其包含SEQ ID NO:19的VH和SEQ ID NO:20的VL,用于治疗患有AML的受治疗者,其中受治疗者具有FLT3-ITD突变。

[0279] 本发明也提供抗CD38抗体,其包含SEQ ID NO:19的VH和SEQ ID NO:20的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的突变。

[0280] 本发明也提供抗CD38抗体,其包含SEQ ID NO:19的VH和SEQ ID NO:20的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的R140Q突变。

[0281] 本发明也提供抗CD38抗体,其包含SEQ ID NO:19的VH和SEQ ID NO:20的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的突变。

[0282] 本发明也提供抗CD38抗体,其包含SEQ ID NO:19的VH和SEQ ID NO:20的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的R882H突变。

[0283] 本发明也提供抗CD38抗体,其包含SEQ ID NO:21的VH和SEQ ID NO:22的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在fms-相关的酪氨酸激酶3(FLT3)中的突变。

[0284] 本发明也提供抗CD38抗体,其包含SEQ ID NO:21的VH和SEQ ID NO:22的VL,用于治疗患有AML的受治疗者,其中受治疗者具有FLT3-ITD突变。

[0285] 本发明也提供抗CD38抗体,其包含SEQ ID NO:21的VH和SEQ ID NO:22的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的突变。

[0286] 本发明也提供抗CD38抗体,其包含SEQ ID NO:21的VH和SEQ ID NO:22的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在异柠檬酸脱氢酶2(IDH2)中的R140Q突变。

[0287] 本发明也提供抗CD38抗体,其包含SEQ ID NO:21的VH和SEQ ID NO:22的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的突变。

[0288] 本发明也提供抗CD38抗体,其包含SEQ ID NO:21的VH和SEQ ID NO:22的VL,用于治疗患有AML的受治疗者,其中受治疗者具有在DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)中的R882H突变。

[0289] 施用/药物组合物

[0290] 在本文所述的本发明的方法中以及在下文所列所有带编号的实施方案的一些实施方案中,抗CD38抗体可在包含抗CD38抗体和药学上可接受的载体的合适药物组合物中提供。载体可为抗CD38抗体与之一起施用的稀释剂、佐剂、赋形剂或溶媒。此类溶媒可以是液

体，诸如水和油，包括那些来源于石油、动物、植物的油或合成的油，诸如花生油、大豆油、矿物油和芝麻油等。例如，可使用0.4％生理盐溶液和0.3％甘氨酸。这些溶液是无菌的，并且通常不含颗粒物。它们可通过熟知的常规灭菌技术(例如过滤)进行灭菌。组合物可根据需要含有药学上可接受的辅助物质，以接近生理条件，诸如pH调节剂和缓冲剂、稳定剂、增稠剂、润滑剂和着色剂等。此类药物制剂中的本发明分子或抗体的浓度可广泛改变，即从小于约0.5重量％，通常到至少约1重量％至多达15重量％或20重量％，并且将根据所选择的具体施用方式，主要基于所需剂量、流体体积、粘度等进行选择。包含其它人蛋白如人血清白蛋白在内的合适的溶媒和制剂在例如Remington:The Science and Practice of Pharmacy，第21版，Troy，D.B.编辑，Lipincott Williams and Wilkins，Philadelphia，PA 2006，第5部分，Pharmaceutical Manufacturing，第691-1092页中有所描述，特别参见第958-989页。

[0291] 在本文所述的本发明的方法中以及下文所列所有带编号的实施方案的一些实施方案中，抗CD38抗体的施用方式可为任何合适的途径，诸如非肠道施用，例如，真皮内、肌肉内、腹膜内、静脉内或皮下、肺、粘膜(口腔、鼻内、阴道内、直肠)或技术人员了解的其它方式，如本领域众所周知的。

[0292] 本文所述的本发明的方法中以及下文所列所有带编号的实施方案的一些实施方案中的抗CD38抗体可通过任何合适的途径施用给患者，例如，通过静脉内(i.v.)输注或推注进行肠胃外施用，肌肉内施用，或皮下施用，或腹膜内施用。可在例如15、30、60、90、120、180或240分钟内，或1、2、3、4、5、6、7、8、9、10、11或12小时内给予静脉内输注。

[0293] 向患有AML的患者给予的剂量足以缓解或至少部分地遏止正在治疗的疾病(“治疗有效量”)，并且有时可为0.005mg/kg至约100mg/kg，例如约0.05mg/kg至约30mg/kg，或约5mg/kg至约25mg/kg，或约4mg/kg，约8mg/kg，约16mg/kg或约24mg/kg，或例如约1、2、3、4、5、6、7、8、9或10mg/kg，但可甚至更高，例如约15、16、17、18、19、20、21、22、23、24、25、30、40、50、60、70、80、90或100mg/kg。

[0294] 也可以给予固定的单位剂量，例如，50、100、200、500或1000mg，或者可以根据患者的体表面积确定剂量，例如，500、400、300、250、200或100mg/m^2。通常可施用介于1和8次之间的剂量(例如，1、2、3、4、5、6、7或8次)以治疗AML，但是可以给予9、10、11、12、13、14、15、16、17、18、19、20或更多次的剂量。

[0295] 本文所述的本发明的方法中以及下文所列所有带编号的实施方案的一些实施方案中的抗CD38抗体可以在一天、两天、三天、四天、五天、六天、一周、两周、三周、一个月、五周、六周、七周、两个月、三个月、四个月、五个月、六个月或更长时间之后重复施用。也可以重复治疗疗程，按照慢性施用一样。重复施用可为相同剂量或不同剂量。例如，本发明的方法中的抗CD38抗体可通过静脉内输注以8mg/kg或以16mg/kg按一周的时间间隔施用8周，接着以8mg/kg或以16mg/kg按每两周一次的方式再施用16周，然后以8mg/kg或以16mg/kg按每四周一次的方式施用。

[0296] 在本文所述的本发明的方法中以及下文所列所有带编号的实施方案的一些实施方案中可通过维持疗法来施用抗CD38抗体，诸如一周一次，为期6个月或更长时间。

[0297] 例如，本文所述的本发明的方法中以及下文所列所有带编号的实施方案的一些实施方案中的抗CD38抗体可以在治疗开始后第1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、

17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39或40天中的至少一天，或者另选地，在第1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19或20周中的至少一周，或其任何组合，采用单次剂量或者每24、12、8、6、4或2小时一次的分次剂量或其任何组合，以约0.1-100mg/kg的量作为日剂量提供，诸如0.5、0.9、1.0、1.1、1.5、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、40、45、50、60、70、80、90或100mg/kg/天。

[0298] 本文所述的本发明的方法中以及下文所列所有带编号的实施方案的一些实施方案中的抗CD38抗体也可以预防性地施用，以降低发展癌症的风险，延迟癌进展中事件的发作，和/或在癌缓解后降低复发的风险。这对于其中肿瘤难以定位而由于其它生物因素已知患有肿瘤的患者可尤其有用。

[0299] 本文所述的本发明的方法中以及下文所列所有带编号的实施方案的一些实施方案中的抗CD38抗体可冻干储存，并在使用之前在合适的载体中复原。此项技术已被证实对常规的蛋白制剂有效，并且可采用熟知的冻干和复原技术。

[0300] 本文所述的本发明的方法中以及下文所列所有带编号的实施方案的一些实施方案中的抗CD38抗体可与全反式视黄酸(ATRA)联合施用。

[0301] 可提供的ATRA剂量为45mg/m^2/天PO或25mg/m^2/天PO。

[0302] 本文所述的本发明的方法中以及下文所列所有带编号的实施方案的一些实施方案中的抗CD38抗体可与达克金联合施用。

[0303] 达克金施用量可为15mg/m^2i.v.3小时，每8小时重复，持续3天，每6周重复，最少4个周期。另选地，达克金施用量可为20mg/m^2i.v.1小时，每日重复，持续5天，并且每4周重复该周期。

[0304] 本文所述的本发明的方法中以及下文所列所有带编号的实施方案的一些实施方案中的抗CD38抗体可与阿糖胞苷和多柔比星联合施用。

[0305] 阿糖胞苷的施用量可为每十二小时2至3g/m^2i.v.1-3小时，最多12剂。

[0306] 多柔比星的施用量可为每21至28天40至60mg/m^2i.v.，或者每21天60至75mg/m^2i.v.一次。

[0307] 抗CD38抗体可连同任何形式的放射疗法和/或外科手术一起施用，放射疗法包括外照射、调强放射疗法(IMRT)和任何形式的放射外科手术，包括伽玛刀、射波刀、直线加速器和组织间放射(例如，植入放射性粒子、GliaSite球囊)。

[0308] 虽然已概括地描述了本发明，但是本发明的实施方案还将在以下的实施例中进一步公开，所述实施例不应理解为限制权利要求的范围。

[0309] 本发明的其它实施方案

[0310] 下文根据本文别处的公开内容陈述了本发明的某些其它实施方案。上文陈述为与本文所公开的发明相关的本发明的实施方案的特征还涉及所有这些其它带编号的实施方案。

[0311] 1.一种用于治疗患有急性髓系白血病(AML)的受治疗者的抗CD38抗体。

[0312] 2.一种用于与第二治疗剂联合治疗患有AML的受治疗者的抗CD38抗体，其中所述第二治疗剂

[0313] a.任选地为阿糖胞苷、佐柔比星、伊达比星、米托蒽醌、羟基脲、地西他滨、克拉屈

滨、氟达拉滨、拓朴替康、依托泊苷6-硫鸟嘌呤、皮质类固醇、泼尼松、地塞米松、甲氨蝶呤、6-巯嘌呤、阿扎胞苷、三氧化二砷或全反式视黄酸;并且/或者

[0314] b.增加CD38的表面表达。

[0315] 3.一种用于治疗患有AML的受治疗者的抗CD38抗体与全反式视黄酸的组合。

[0316] 4.一种用于治疗患有AML的受治疗者的抗CD38抗体与地西他滨的组合。

[0317] 5.一种用于治疗患有AML的受治疗者的抗CD38抗体与阿糖胞苷和/或多柔比星的组合。

[0318] 6.根据实施方案1或2所述的所用抗CD38抗体、或根据实施方案3-5所述的组合,其中所述抗CD38抗体与包含SEQ ID NO:4的重链可变区(VH)和SEQ ID NO:5的轻链可变区(VL)的抗体竞争结合CD38。

[0319] 7.根据实施方案1、2或6所述的所用抗CD38抗体、或根据实施方案3-6所述的组合,其中所述抗CD38抗体通过细胞凋亡来诱导杀伤表达CD38的AML细胞。

[0320] 8.根据实施方案1、2、6或7所述的所用抗CD38抗体、或根据实施方案3-7所述的组合,其中所述抗CD38抗体结合于人CD38(SEQ ID NO:1)的区域SKRNIQFSCKNIYR(SEQ ID NO:2)和区域EKVQTLEAWVIHGG(SEQ ID NO:3)。

[0321] 9.根据实施方案1、2、6-8所述的所用抗CD38抗体、或根据实施方案3-8所述的组合,其中所述抗CD38抗体:

[0322] a.具有IgG1、IgG2、IgG3或IgG4同种型;

[0323] b.具有岩藻糖含量为约50%、40%、45%、40%、35%、30%、25%、20%、15%、14%、13%、12%、11%、10%、9%、8%、7%、6%、5%、4%、3%、2%、1%或0%的双分枝聚糖结构;或者

[0324] c.当根据EU索引对残基进行编号时,包含在所述抗体Fc中的氨基酸位置256、290、298、312、356、330、333、334、360、378或430处的置换。

[0325] 10.根据实施方案1、2、6-9所述的所用抗CD38抗体、或根据实施方案3-9所述的组合,其中所述抗CD38抗体包含

[0326] a.分别为SEQ ID NO:6、7和8的重链互补决定区(HCDR)1(HCDR1)、2(HCDR2)和3(HCDR3)序列;

[0327] b.分别为SEQ ID NO:9、10和11的轻链互补决定区(LCDR)1(LCDR1)、2(LCDR2)和3(LCDR3)序列;

[0328] c.分别为SEQ ID NO:6、7、8、9、10和11的HCDR1、HCDR2、HCDR3、LCDR1、LCDR2和LCDR3序列;

[0329] d.SEQ ID NO:4的重链可变区(VH)和SEQ ID NO:5的轻链可变区(VL);

[0330] e.包含与SEQ ID NO:12的氨基酸序列具有95%、96%、97%、98%或99%同一性的氨基酸序列的重链和包含与SEQ ID NO:13的氨基酸序列具有95%、96%、97%、98%或99%同一性的氨基酸序列的轻链;或

[0331] f.SEQ ID NO:12的重链和SEQ ID NO:13的轻链。

[0332] 11.根据实施方案1、2、6-10所述的所用抗CD38抗体、或根据实施方案3-10所述的组合,其中伴有至少一种遗传异常的AML、伴有多系病变的AML、治疗相关性AML、未分化型AML、微成熟型AML、成熟型AML、急性髓单核细胞白血病、急性单核细胞白血病、急性红白血

病、急性巨核细胞白血病、急性嗜碱性白血病、急性全髓纤维化或髓系肉瘤。

[0333] 12.根据实施方案1、2、6-11所述的所用抗CD38抗体、或根据实施方案3-11所述的组合，其中所述抗CD38抗体作为缓解诱导、缓解后或维持疗法来施用。

[0334] 13.根据实施方案1、2、6-12所述的所用抗CD38抗体、或根据实施方案3-12所述的组合，其中所述至少一种遗传异常是染色体8和21之间的易位，染色体16中的易位或倒位，染色体15和17之间的易位，染色体11的改变，或者fms-相关的酪氨酸激酶3(FLT3)、核仁磷酸蛋白(NPM1)、异柠檬酸脱氢酶1(IDH1)、异柠檬酸脱氢酶2(IDH2)、DNA(胞嘧啶-5)-甲基转移酶3(DNMT3A)、CCAAT/增强子结合蛋白α(CEBPA)、U2小核RNA辅助因子1(U2AF1)、zeste 2多梳抑制复合物2亚基的增强子(EZH2)、染色体1A的结构维持(SMC1A)或染色体3的结构维持(SMC3)中的突变。

[0335] 14.根据实施方案1、2、6-13所述的所用抗CD38抗体、或根据实施方案3-13所述的组合，其中所述至少一种遗传异常是IDH1中的易位t(8;21)(q22;q22)、倒位inv(16)(p13;q22)、易位t(16;16)(p13;q22)、易位t(15;17)(q22;q12)、突变FLT3-ITD、突变R132H或R100Q/R104V/F108L/R119Q/I130V或者IDH2中的突变R140Q或R172。

[0336] 15.根据实施方案1、2、6-14所述的所用抗CD38抗体、或根据实施方案3-14所述的组合，其中所述抗CD38抗体和至少一种治疗剂同时、依序或分开施用。

[0337] 16.根据实施方案1、2、6-15所述的所用抗CD38抗体、或根据实施方案3-15所述的组合，其中

[0338] a.所述受治疗者进一步经历放射疗法治疗或已经历放射疗法治疗；或者

[0339] b.所述受治疗者已接受造血干细胞移植。

[0340] 实施例

[0341] 实施例1：达雷木单抗在AML细胞系中的疗效

[0342] 若干AML细胞系用于评估CD38的表面表达和达雷木单抗在诱导AML细胞杀伤中的可能疗效。评估补体抑制蛋白(CIP)CD46、CD55和CD59在AML细胞系中的表达以评估CIP和CDC表达之间的可能关联。

[0343] 方法：

[0344] ADCC

[0345] 使用AML肿瘤细胞系和外周血单核细胞(PBMC)作为效应细胞进行体外ADCC测定，细胞比率为50:1。将100μl靶(肿瘤)细胞(1×10^4个细胞)加入96孔U-底板的孔中。在带有或不带有抗体的情况下另加入100μl，并且板在加入效应细胞(PBMC)前于室温(RT)下孵育30分钟。将浓度为6.66×10^6个细胞/ml的75μl PBMC加入板的孔中，并且板在37℃下孵育6小时。板以250g离心4分钟，每个孔移除50μl上清液并使用CellTiter-Glo®测定法(Promega)测量细胞裂解。

[0346] CDC

[0347] 收获靶细胞并调节至80×10^4个细胞/ml的浓度。将12μl靶细胞加到96孔板的孔中，并且将系列稀释的抗体加到孔中。孔孵育15分钟，之后加入人血清高补体，最终浓度为10％。反应混合物在37℃下孵育21/2小时，并且使用CellTiter-Glo®测定法(Promega)测量细胞裂解。

[0348] 细胞凋亡

[0349] 在存在或不存在兔抗人IgG(10μg/ml;F(ab')2Fcγ-特异性)的情况下,将1ml靶细胞(5×10^{5}个细胞/ml)加入24孔板的孔中,同时加入测试抗体(1μg/ml)。细胞孵育22小时(5%CO2,37℃)。然后收获细胞(1000rpm,5分钟)并用PBS洗涤两次(1000rpm,5分钟)。根据制造商的说明书,将细胞重悬在250μl结合缓冲液(Annexin-V细胞凋亡试剂盒,BD Biosciences)中,随后进行流式细胞术分析。

[0350] 通过早期和晚期细胞凋亡(图1A和图1B中的Q2和Q3)测量细胞凋亡。

[0351] CD38、CD46、CD55和CD59的表面表达

[0352] 通过流式细胞术分析受体表达。每个细胞的CD38受体数使用MESF试剂盒,利用PE-标记的抗CD38抗体(R&D Systems)进行估计。受体数如下计算:比MESF/ABC=MESF/ABC(测试抗体)-MESF/ABC(同种型对照抗体)。

[0353] CD46、CD55和CD59的表面表达使用FITC抗人CD46、PE-抗人CD55和PE-抗人CD59抗体(Beckton Dickinson)进行检测,结果表达为中位荧光强度(MFI)。

[0354] 结果

[0355] 表1示出实验结果。图1示出在无(图1A)或有(图1B)交联抗体的情况下,达雷木单抗在NB-4细胞系中诱导的细胞凋亡的代表性流式细胞术结果。在该细胞系中,达雷木单抗诱导细胞凋亡至相似的程度,与交联剂存在与否无关(19.2%对18.3%)。

[0356] 在AML细胞系中,达雷木单抗不诱导显著的ADCC或CDC;相反地;达雷木单抗诱导通过细胞凋亡的AML细胞杀伤。此外,在CD38表达与ADCC和CDC程度之间未观察到直接的相关性。补体抑制蛋白(CIP)(CD46、CD55和CD59)的含量经评估以测定是否这些蛋白响应达雷木单抗而影响CDC,但是在CDC和CIP表达之间未观察到直接的相关性。

[0357] 表1:

[0358]

细胞系	CD38 #/细胞	CD46 MFI	CD55 MFI	CD59 MFI	细胞凋亡	CDC	ADCC
HL-60	64.50	ND	ND	ND	ND	ND	ND
Kasumi-1	120.2	ND	ND	ND	ND	ND	ND
ML-2	1,253.27	21.53	195.2	0.98	5%	0%	6.30%
MOLM-13	5,634.29	35.53	173.2	9.45	10%-15%	0%	9.40%
MOLM-16	52,461.11	42.18	886.4	350.42	20-30%	5%	18.20%
MV-4-11	5,700.05	207.17	395.42	43.94	10%-12%	0%	2.30%
NB4	9,370.73	58.25	345.4	66.2	18%	4%	18.30%
THP-1	39,488.19	58.7	375	27.1	5-7%	5%	11.30%
ND: 未完成							
MFI: 平均荧光强度							

[0359] 实施例2:ATRA诱导在AML细胞上的CD38表达

[0360] ATRA对CD38表面表达的效应在NB-4AML细胞系中评估。在存在或不存在10nM或100nM ATRA的情况下,将肿瘤细胞在37℃下孵育24小时。在孵育24小时后,收获细胞并染色CD38。在NB-4细胞系中ATRA诱导CD38受体增加~10倍。CD38表面表达使用FACS,利用PE-标记的抗CD38抗体(R&D Systems)进行评估(表2)。

[0361] 表2:

[0362]

治疗	PE-CD38分子/细胞
DMSO	17238
10nMATRA	185737
100nMATRA	210570

[0363] 实施例3:达雷木单抗在来源于患者的异种移植(PDX)模型中的疗效

[0364] 方法

[0365] 研究使用患者肿瘤模型AML3406、AML 7577和AML 8096。

[0366] AML3406模型:患者肿瘤细胞为FLT-3ITD阳性。患者有真性红细胞增多症病史,并且接受伊达比星/阿糖胞苷用于诱导化学治疗。患者也接受Hudrea®(羟基脲)治疗。

[0367] AML 7577模型:白血病细胞收集自患有AML(FAB亚型M5)的69岁男性。患者具有正常核型和以下突变:IDH2(R140Q);FLT3-ITD;DNMT3A R882H、NPM1、CEBPA插入(SNP)。患者有真性红细胞增多症病史,并且接受伊达比星/阿糖胞苷用于诱导化学治疗。患者也接受Hudrea®(羟基脲)治疗。

[0368] AML 8096模型:白血病细胞收集自患有AML(FAB亚型M2)的21岁男性。白血球计数为$20\times10e^{9}$/L,其中70%为母细胞。患者具有正常核型,带有野生型TP53、FLT3、NPM1、以及在CEBPA exon1中的插入570-587、3GCACCC>4GCACCC。患者有真性红细胞增多症病史,并且接受伊达比星/阿糖胞苷用于诱导化学治疗。患者也接受Hudrea®(羟基脲)治疗。

[0369] 5百万AML MNC经T细胞清除并经由尾静脉移植入6-8周龄的亚致死剂量辐照NSG小鼠(每组n=10)。在植入后4至6周,收集每只小鼠的骨髓穿刺液并通过流式细胞术进行分析,测定白血病植入物的含量(%人$CD45^{+}CD33^{+/-}$细胞)。基于植入水平,小鼠用IgG1或达雷木单抗(DARA,预剂量0.5mg/kg)随机化并调理。24小时后,小鼠未经治疗(Ctrl)或者经DARA或IgG1单独治疗连续5周(i.p,10mg/kg,每周一次)。在最后一次治疗后2-3天,杀死小鼠并收集骨髓、脾、外周血和血浆用于分析。进行流式细胞术以评估在植入3个AML患者的BM、SPL和PB的NSG小鼠(AML 3406模型:图2A,AML 7577模型:图2B,AML 8096模型:图2C)中的人$CD45^{+}CD33^{+}$细胞百分比以及一个代表性AML患者的骨髓(图3A)、脾(图3B)和外周血(图3C)中的人$CD45^{+}CD33^{+}$细胞的绝对数。

[0370] 结果

[0371] 图2A、图2B和图2C分别示出达雷木单抗在AML 3406模型、AML 7577模型和AML 8096模型中的疗效,它们通过骨髓、脾或外周血中白血病$CD45^{+}CD33^{+}$细胞减少%进行评估。达雷木单抗在AML 3406模型(图2A)中的脾和外周血中、在AML 7577模型(图2B)的外周血中、以及在AML 8096模型(图2C)中的脾中减小肿瘤负荷。

[0372] 达雷木单抗的疗效也通过测量AML 3406模型中的骨髓(图3A)、脾(图3B)和血液(图3C)中的达雷木单抗诱导的总白血病负荷减小进行评估。达雷木单抗显著减小AML 3406模型中的脾(图3B)和外周血(图3C)中的总白血病负荷。

[0373] 实施例4:达雷木单抗对AML母细胞上的CD38表达的效应

[0374] 达雷木单抗对在白血病母细胞上的CD38表达的效应在用达雷木单抗或利用PE标记抗CD38抗体(R&D Systems)的同种型对照治疗5周后,在实施例3所述的一个代表性AML模型中进行评估。

[0375] 结果

[0376] 图4A示出达雷木单抗治疗减少在骨髓、脾和外周血中的白血病母细胞(CD45$^+$CD33$^+$阳性细胞)上的CD38表达。图4B示出在治疗5周后CD38-阳性AML母细胞的减少百分比。

[0377] 实施例5:达雷木单抗组合疗法在来源于患者的异种移植(PDX)模型中的疗效

[0378] 在治疗5周后评估达雷木单抗与达克金或阿糖胞苷和多柔比星的组合的疗效。

[0379] 5百万AML MNC经T细胞清除并经由尾静脉移植入6-8周龄的NSG小鼠(每组n＝10)。在植入后4至6周,收集每只小鼠的骨髓穿刺液并通过流式细胞术进行分析,测定白血病植入物的含量(%人CD45$^+$CD33$^{+/-}$细胞)。基于植入水平,小鼠用IgG1或DARA(预剂量0.5mg/kg)等几率随机化并调理。24小时后,小鼠用IgG1单独治疗(i.p,10mg/kg),每周一次,持续五周,用DARA单独治疗(i.p,10mg/kg),每周一次,持续五周,用地西他滨单独治疗(DAC)(0.5mg/kg/天,i.p.连续3天),持续五周,用DAC+DARA治疗(每周将包括连续3天的DAC治疗,后接2天的DARA治疗),在使用或不使用DARA的情况下用阿糖胞苷(i.v,50mg/kg)和多柔比星(i.v,1.5mg/kg)(多柔比星(i.v,1.5mg/kg)连续治疗3天加阿糖胞苷(50mg/kg)治疗3天)的组合治疗。在最后一次治疗后2-3天,杀死小鼠并收集骨髓、脾、外周血和血浆用于分析。进行流式细胞术以评估在一个AML患者植入的NSG小鼠中的骨髓(图5A)、脾(图5B)和外周血(图5C)中的人CD45$^+$CD33$^+$细胞百分比。

[0380] 在用指示药物治疗5周后,CD38表达(表示为平均荧光强度,MFI)在骨髓(图6A)、脾(图6B)和外周血(图6C)中进行评估。

序列表

<110> Doshi, Parul
Danet-Desnoyers, Gwenn
Dos Santos, Cedric
Sasser, Amy
Shan, Xiaochuan

<120> 用于治疗急性髓系白血病的抗 CD38 抗体

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<213> 智人 (Homo sapiens)

<400> 3

Glu Lys Val Gln Thr Leu Glu Ala Trp Val Ile His Gly Gly
1 5 10

<210> 4
<211> 122
<212> PRT
<213> 人工序列

[0003]

<220>
<223> 抗体 VH

<400> 4

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Phe Thr Phe Asn Ser Phe
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Gly Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Phe Cys

85 90 95

Ala Lys Asp Lys Ile Leu Trp Phe Gly Glu Pro Val Phe Asp Tyr Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 5
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 抗体 VL

<400> 5

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
[0004] 20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 6
<211> 5
<212> PRT
<213> 人工序列

<220>
<223> 抗体 HCDR1

<400> 6

Ser Phe Ala Met Ser
1 5

<210> 7
<211> 17
<212> PRT
<213> 人工序列

<220>
<223> 抗体 HCDR2

<400> 7

Ala Ile Ser Gly Ser Gly Gly Gly Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

[0005]

<210> 8
<211> 13
<212> PRT
<213> 人工序列

<220>
<223> 抗体 HCDR3

<400> 8

Asp Lys Ile Leu Trp Phe Gly Glu Pro Val Phe Asp Tyr
1 5 10

<210> 9
<211> 11
<212> PRT
<213> 人工序列

<220>
<223> 抗体 LCDR1

<400> 9

Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala

1 5 10

<210> 10
<211> 7
<212> PRT
<213> 人工序列

<220>
<223> 抗体 LCDR2

<400> 10

Asp Ala Ser Asn Arg Ala Thr
1 5

<210> 11
<211> 10
<212> PRT
<213> 人工序列

<220>
<223> 抗体 LCDR3

[0006] <400> 11

Gln Gln Arg Ser Asn Trp Pro Pro Thr Phe
1 5 10

<210> 12
<211> 452
<212> PRT
<213> 人工序列

<220>
<223> 抗体 HC

<400> 12

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Phe Thr Phe Asn Ser Phe
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

```
Ser Ala Ile Ser Gly Ser Gly Gly Gly Thr Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Phe Cys
                85                  90                  95

Ala Lys Asp Lys Ile Leu Trp Phe Gly Glu Pro Val Phe Asp Tyr Trp
            100                 105                 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
        115                 120                 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
    130                 135                 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145                 150                 155                 160
```

[0007]

```
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
                165                 170                 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
            180                 185                 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
        195                 200                 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
    210                 215                 220

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225                 230                 235                 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
                245                 250                 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
```

260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
275 280 285

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
290 295 300

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305 310 315 320

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
325 330 335

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
340 345 350

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
355 360 365

[0008]

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
370 375 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385 390 395 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
405 410 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
420 425 430

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
435 440 445

Ser Pro Gly Lys
450

<210> 13
<211> 214

<212> PRT
<213> 人工序列

<220>
<223> 抗体 LC

<400> 13

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

[0009]

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr

180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 14
<211> 4
<212> PRT
<213> 智人 (Homo sapiens)

<400> 14

Val Gln Leu Thr
1

<210> 15
<211> 122
<212> PRT
<213> 人工序列

[0010]

<220>
<223> 抗体 VH

<400> 15

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Phe Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Val Ile Pro Phe Leu Gly Ile Ala Asn Ser Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Asp Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys

85 90 95

Ala Arg Asp Asp Ile Ala Ala Leu Gly Pro Phe Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser
115 120

<210> 16
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 抗体 VL

<400> 16

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
[0011] 20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Tyr Pro Arg
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 17
<211> 122
<212> PRT
<213> 人工序列

<220>
<223> 抗体 VH

<400> 17

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1 5 10 15

Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Ser Asn Tyr
20 25 30

Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Ile Ile Tyr Pro His Asp Ser Asp Ala Arg Tyr Ser Pro Ser Phe
50 55 60

Gln Gly Gln Val Thr Phe Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65 70 75 80

[0012]

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg His Val Gly Trp Gly Ser Arg Tyr Trp Tyr Phe Asp Leu Trp
100 105 110

Gly Arg Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 18
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 抗体 VL

<400> 18

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

[0013]

<210> 19
<211> 120
<212> PRT
<213> 人工序列

<220>
<223> 抗体 VH

<400> 19

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Tyr Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Ser Gly Asp Pro Ser Asn Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Leu Pro Leu Val Tyr Thr Gly Phe Ala Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 20
<211> 109
<212> PRT
<213> 人工序列

<220>
<223> 抗体 VL

<400> 20

Asp Ile Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1 5 10 15

[0014]

Thr Ala Arg Ile Ser Cys Ser Gly Asp Asn Leu Arg His Tyr Tyr Val
20 25 30

Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
35 40 45

Gly Asp Ser Lys Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
50 55 60

Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Glu
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Gln Thr Tyr Thr Gly Gly Ala Ser Leu
85 90 95

Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105

<210> 21
<211> 120

<212> PRT
<213> 人工序列

<220>
<223> 抗体 VH

<400> 21

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Ala Lys Pro Gly Thr
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Trp Met Gln Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Thr Ile Tyr Pro Gly Asp Gly Asp Thr Gly Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Lys Thr Val Tyr
65 70 75 80

[0015]

Met His Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Asp Tyr Tyr Gly Ser Asn Ser Leu Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Ser Val Thr Val Ser Ser
115 120

<210> 22
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 抗体 VL

<400> 22

Asp Ile Val Met Thr Gln Ser His Leu Ser Met Ser Thr Ser Leu Gly
1 5 10 15

```
Asp Pro Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Ser Thr Val
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Arg Arg Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Tyr Arg Tyr Ile Gly Val Pro Asp Arg Phe Thr Gly
    50                  55                  60
```

[0016]

```
Ser Gly Ala Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val Gln Ala
65                  70                  75                  80

Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln His Tyr Ser Pro Pro Tyr
                85                  90                  95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105
```

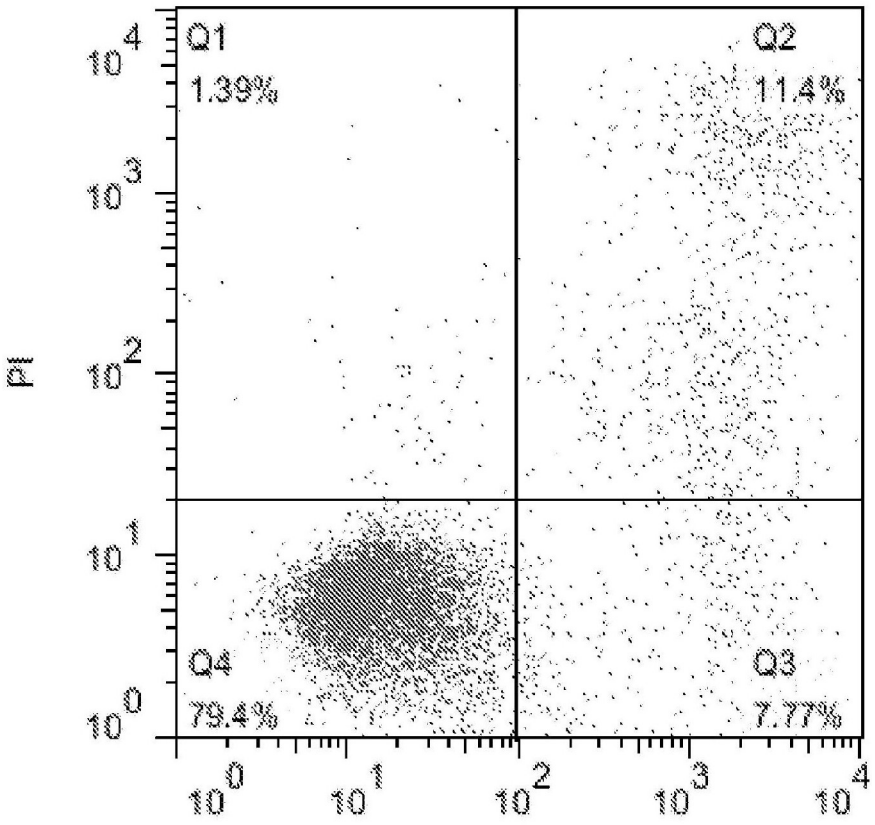

图1A

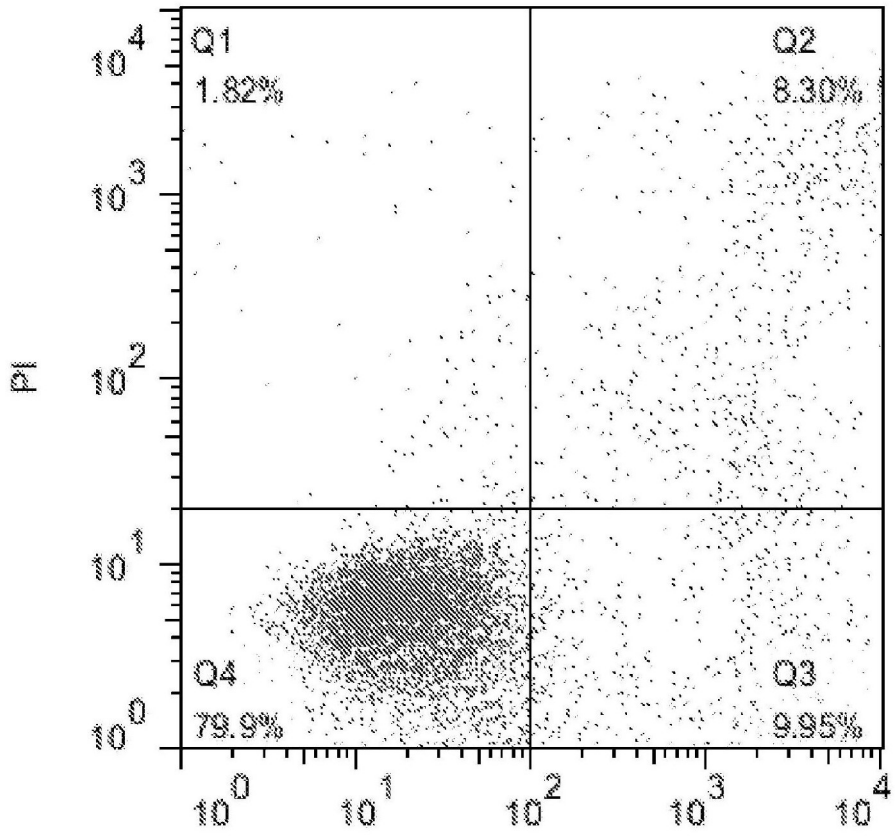
Q1
1.82%
Q2
8.30%
Q4
79.9%
Q3
9.95%
PI
10^4
10^3
10^2
10^1
10^0
10^0
10^1
10^2
10^3
10^4
膜联蛋白V:FITC

图1B

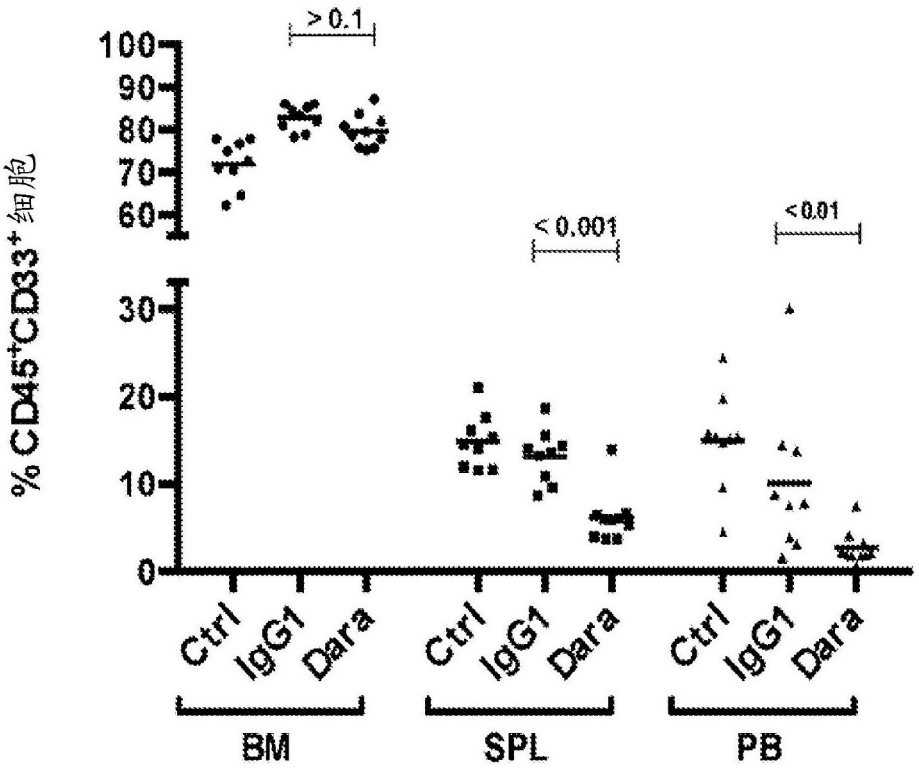
% CD45+CD33+细胞
100
90
80
70
60
30
20
10
0
> 0.1
< 0.001
< 0.01
Ctrl
IgG1
Dara
BM
SPL
PB

图2A

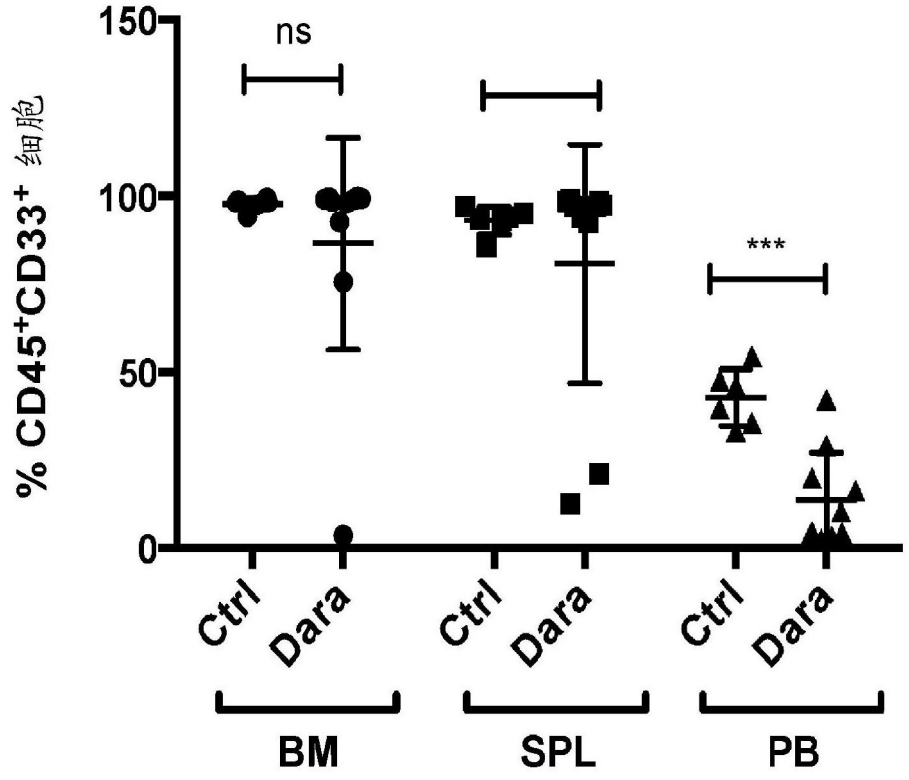
% CD45+CD33+ 细胞
150
100
50
0
ns
***
Ctrl
Dara
BM
SPL
PB

图2B

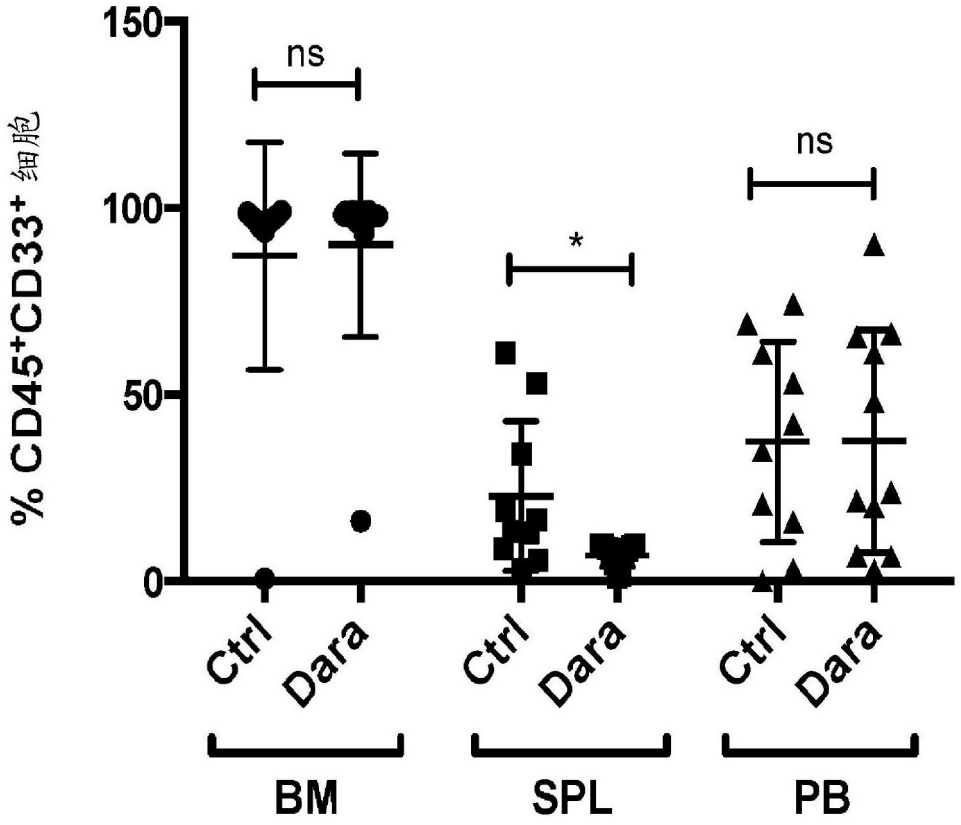
150
100
50
0
% CD45+CD33+ 细胞
ns
*
ns
Ctrl
Dara
Ctrl
Dara
Ctrl
Dara
BM
SPL
PB

图2C

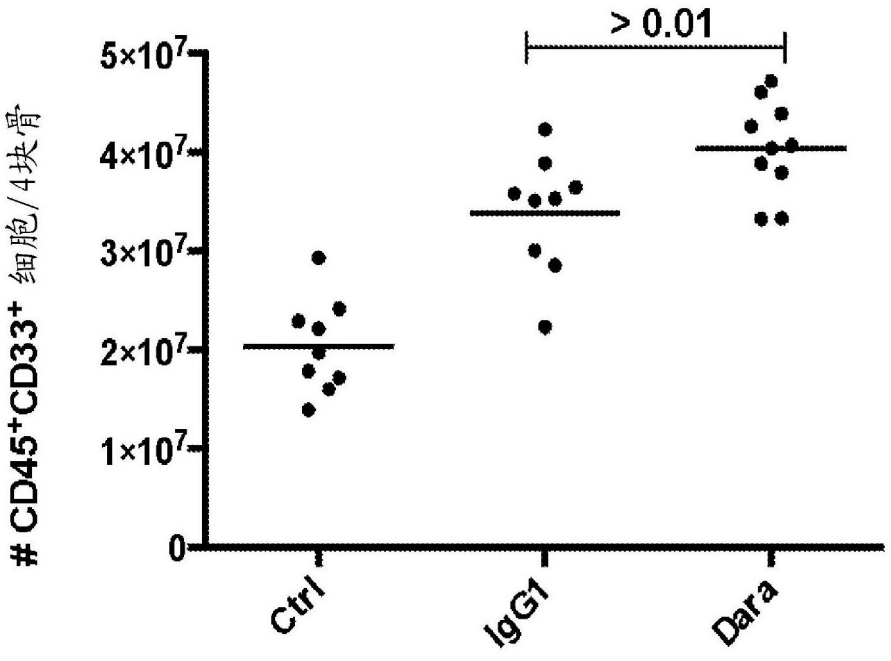
> 0.01
5×10^7
4×10^7
3×10^7
2×10^7
1×10^7
0
CD45+CD33+ 细胞/4块骨
Ctrl
IgG1
Dara

图3A

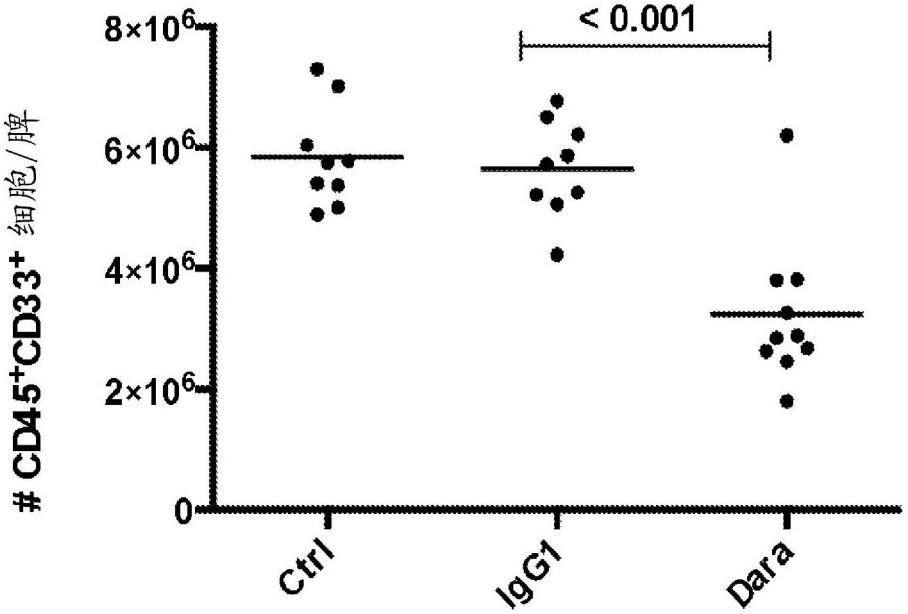
8×10^6
6×10^6
4×10^6
2×10^6
0
CD45+CD33+ 细胞/脾
< 0.001
Ctrl
IgG1
Dara

图3B

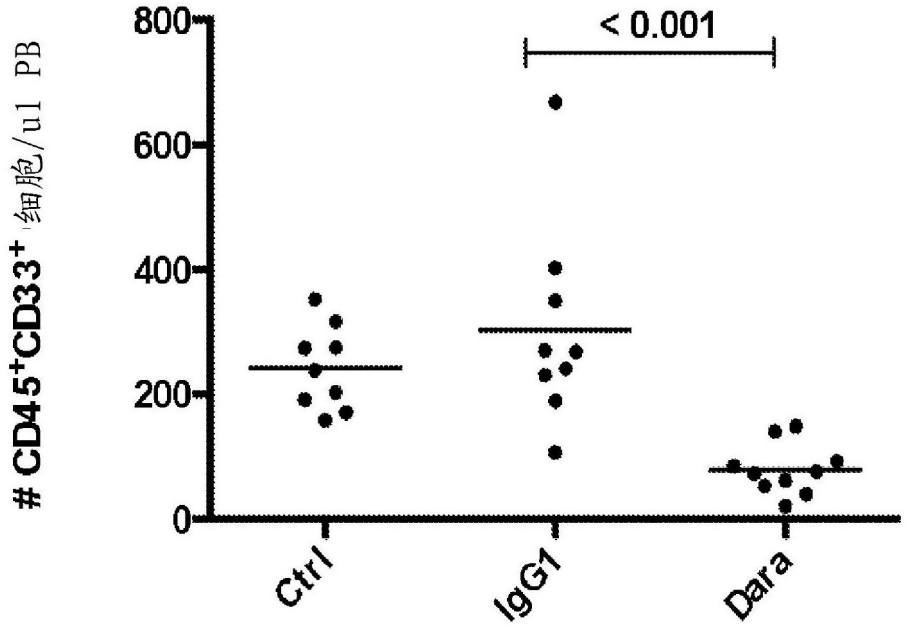
800
600
400
200
0
CD45+CD33+ 细胞/ul PB
< 0.001
Ctrl
IgG1
Dara

图3C

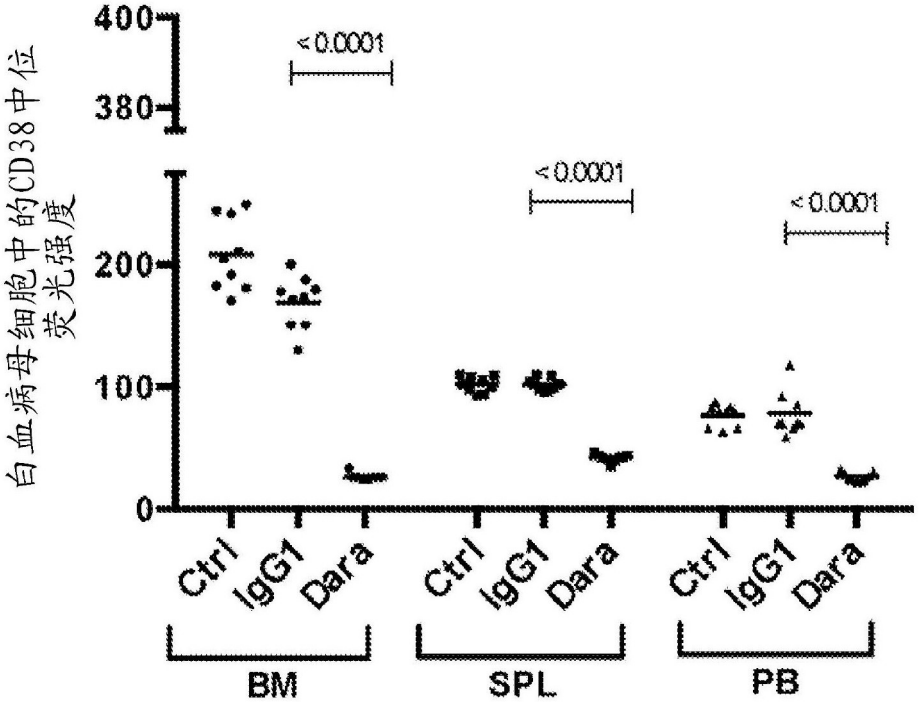
白血病母细胞中的CD38中位荧光强度
400
380
200
100
0
<0.0001
<0.0001
<0.0001
Ctrl
IgG1
Dara
BM
SPL
PB

图4A

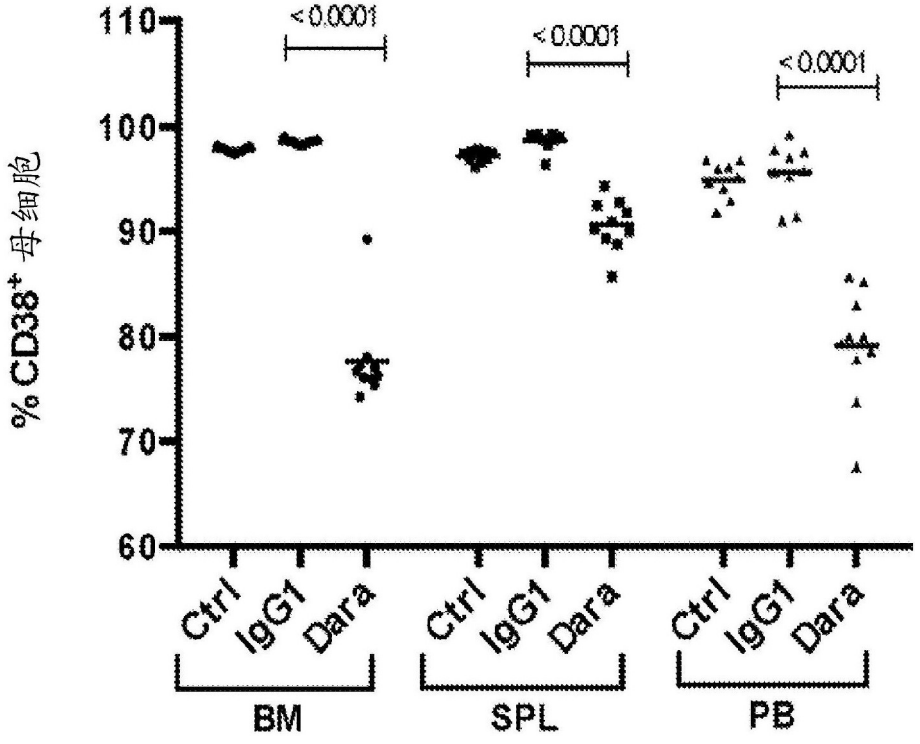
% CD38+ 母细胞
110
100
90
80
70
60
<0.0001
<0.0001
<0.0001
Ctrl
IgG1
Dara
BM
SPL
PB

图4B

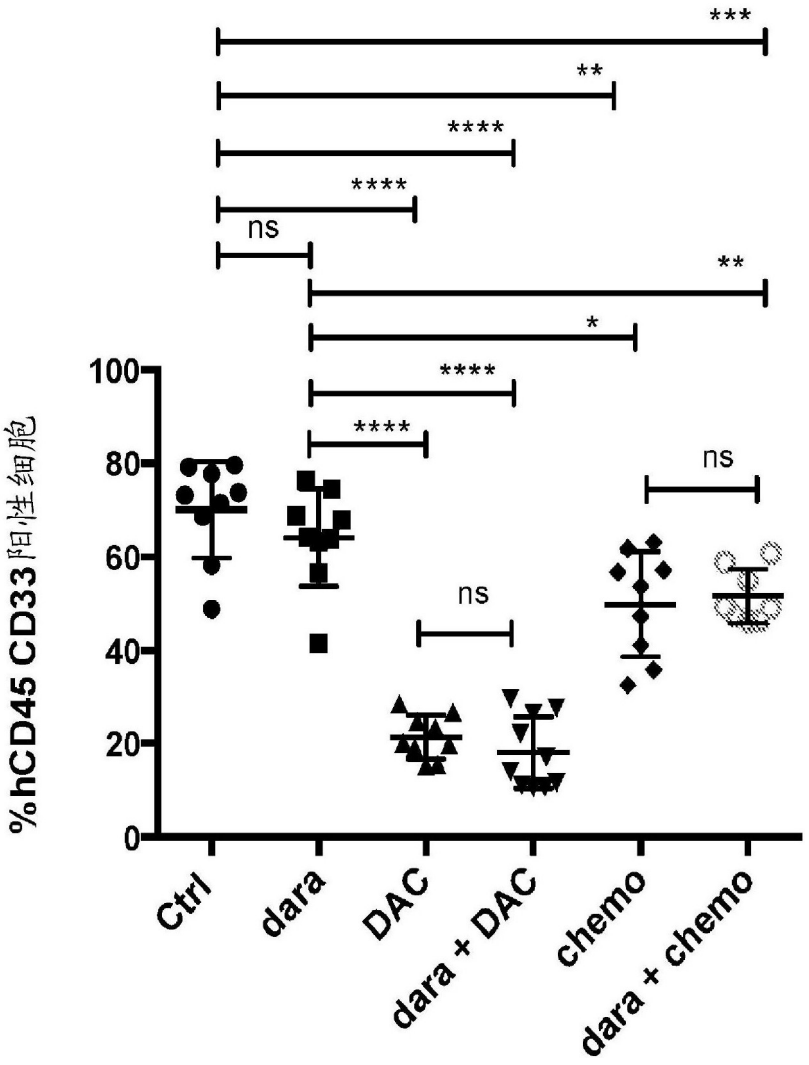
***
**
****
****
ns
**
*
****
****
ns
ns
100
80
60
40
20
0
%hCD45 CD33阳性细胞
Ctrl
dara
DAC
dara + DAC
chemo
dara + chemo

图5A

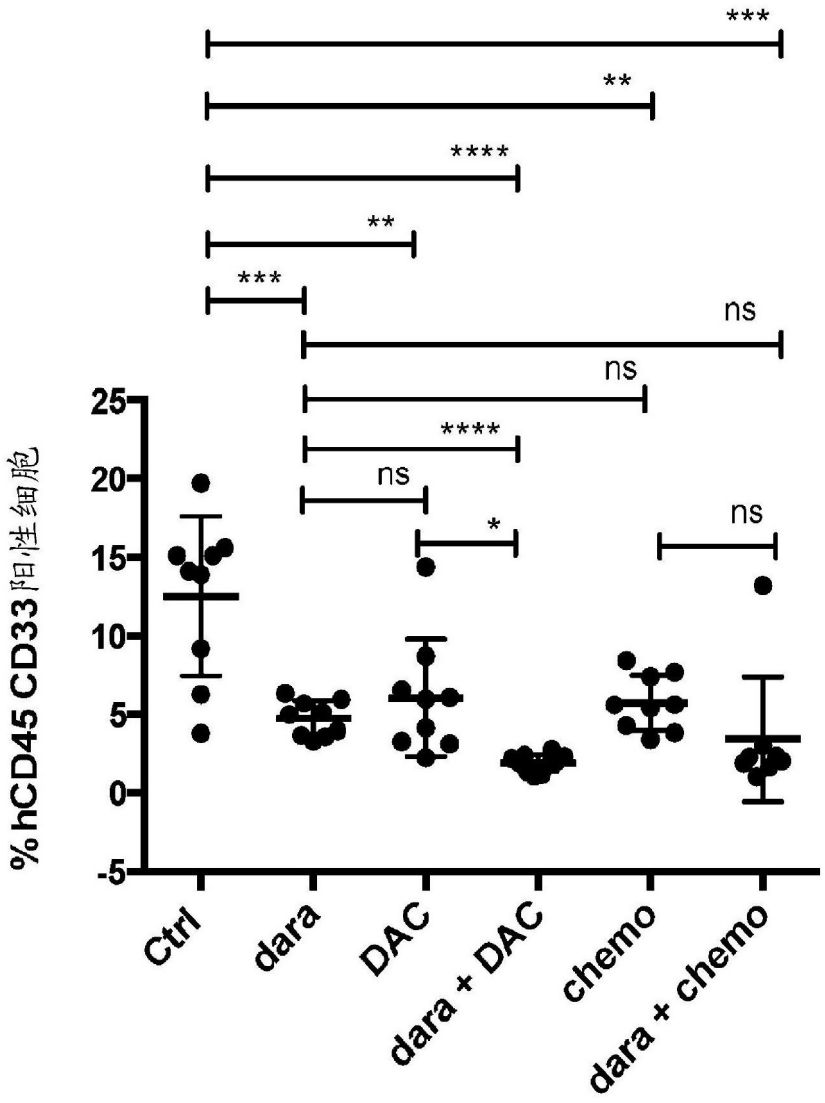
***
**
****
**
***
ns
ns
****
ns
*
ns
% hCD45 CD33阳性细胞
25
20
15
10
5
0
-5
Ctrl
dara
DAC
dara + DAC
chemo
dara + chemo

图5B

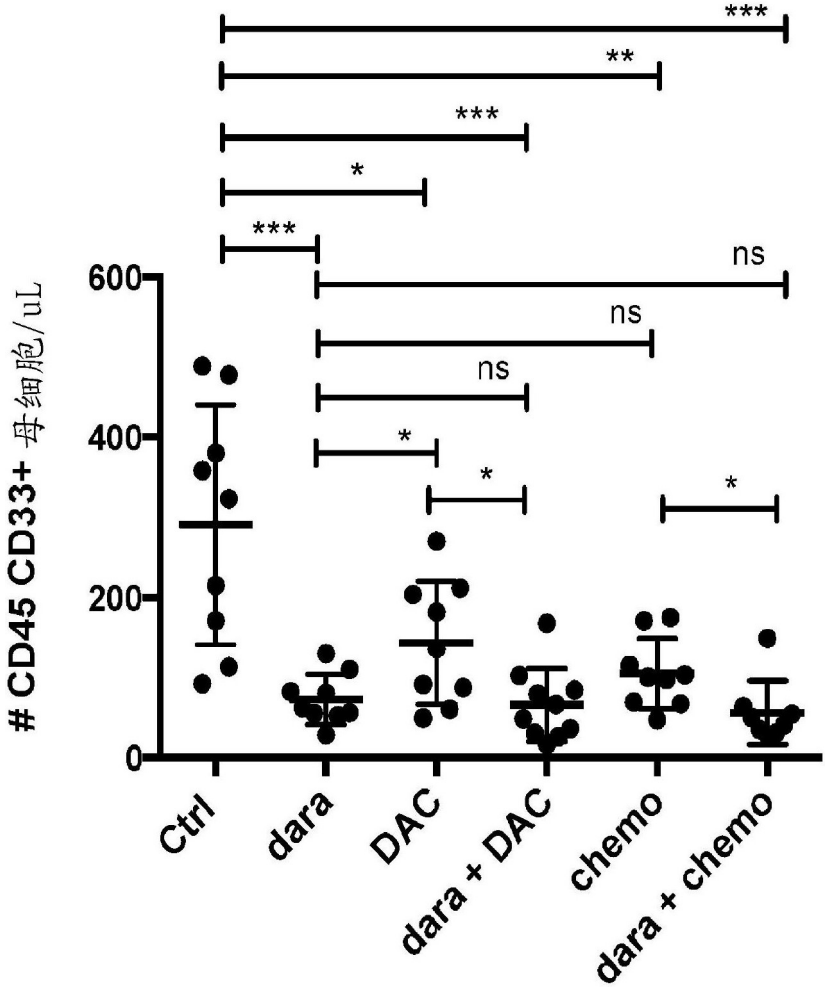
***
**
***
*
***
ns
ns
ns
*
*
*
600
400
200
0
CD45 CD33+ 母细胞/uL
Ctrl
dara
DAC
dara + DAC
chemo
dara + chemo

图5C

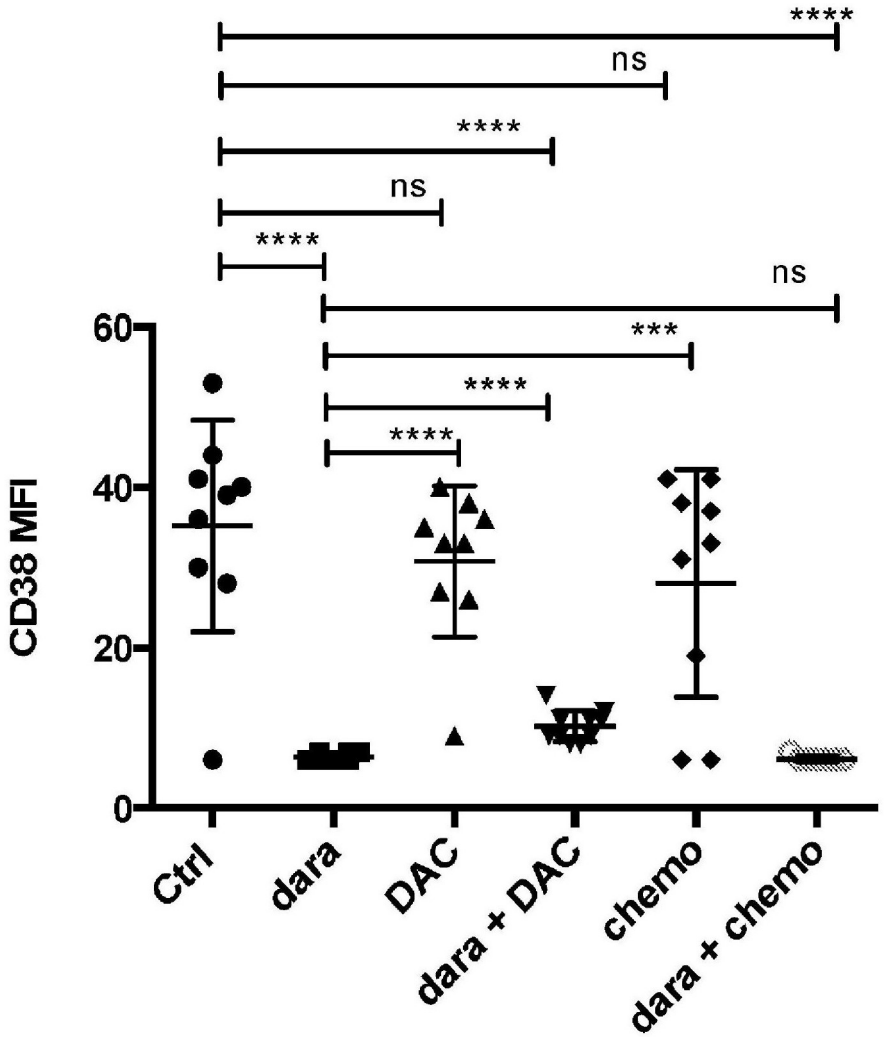
****
ns
****
ns
****
ns
***
****
****
CD38 MFI
60
40
20
0
Ctrl
dara
DAC
dara + DAC
chemo
dara + chemo

图6A

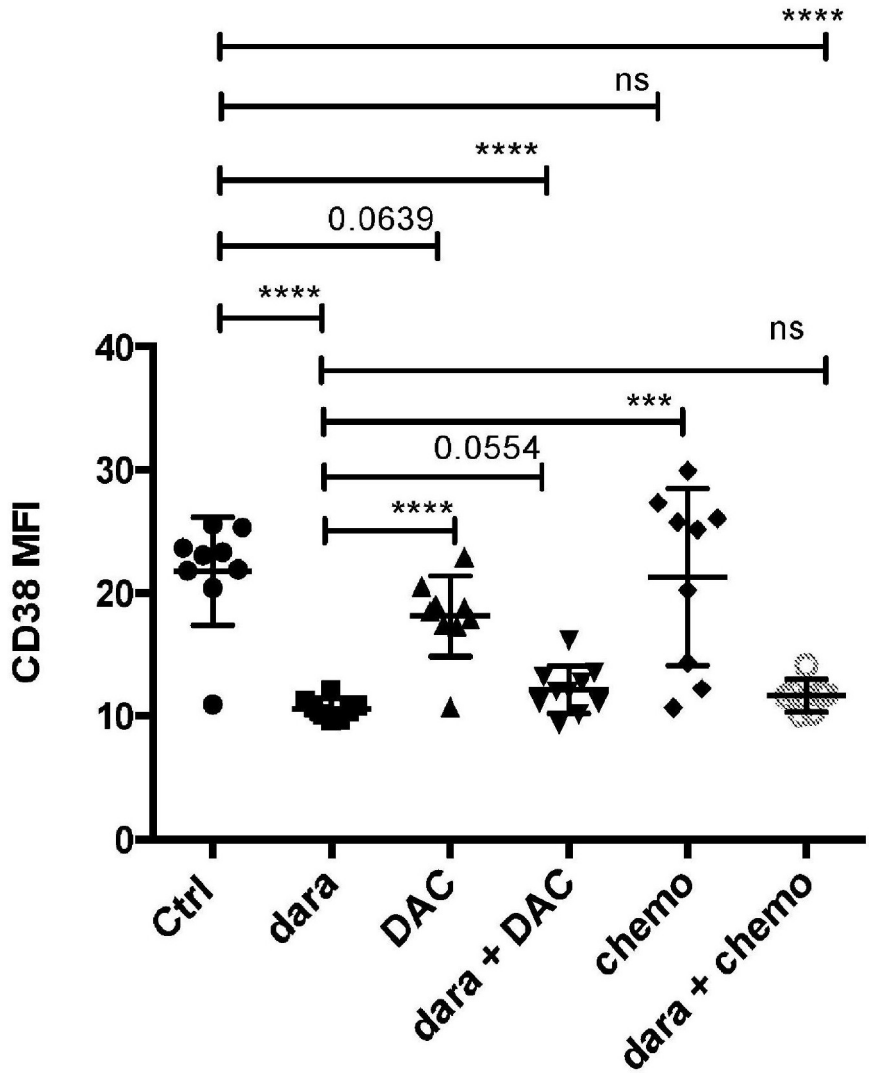
****
ns
****
0.0639
****
ns
***
0.0554
****
CD38 MFI
40
30
20
10
0
Ctrl
dara
DAC
dara + DAC
chemo
dara + chemo

图6B

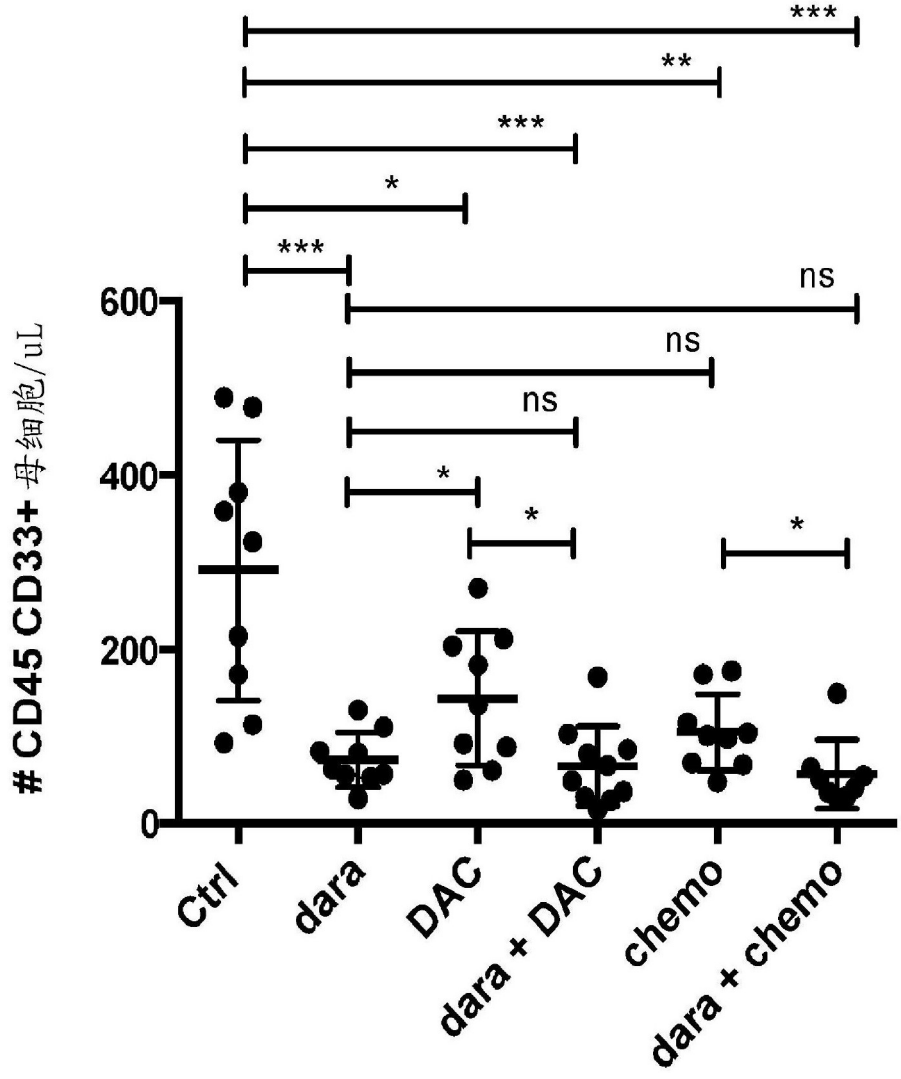
***
**
***
*
***
ns
ns
ns
*
*
*
600
400
200
0
CD45 CD33+ 母细胞/uL
Ctrl
dara
DAC
dara + DAC
chemo
dara + chemo

图6C